REMARKS/ARGUMENTS

Favorable consideration of the present application and the above claims is respectfully requested.

In the present amendment, Applicant has amended the specification to correct the domestic priority claim for the subject application and to correct other informalities objected to by the Examiner. No new matter is added by these amendments to the specification.

Claims 1 – 22 are pending. Independent claims 1, 2, 3, 4, and 10 are amended claim the unique features of Applicant's invention, as discussed in detail below. Support for the amendments to Claims 1, 2, 3, 4 and 10 is found in the specification on page 5, lines 12-31; page 6, lines 1-18; page 7, lines 3-21; page 8, lines 6-26; and Figure 8.

Claim 6 is amended to correct an inadvertent spelling error; the word "fluorine" is replaced by the intended word – fluorene--.

No new matter is added by the amendments identified above.

Applicants now respond to the detailed action starting with a statement of Applicants' invention, as now claimed: Applicant's invention increases the data storage capacity in a data storage medium, by at least 50%, using a ternary encoding scheme rather than a binary encoding scheme, wherein both image writing and reading are accomplished via near-IR two-photon excitation of polymer films containing a reactive dye (fluorophore) and a photo-acid generator. Prior to Applicant's invention, it was not known that a chemical process would yield image formation within a photosensitive polymeric film containing a photo-acid generator and an acid-sensitive fluorophore, which allows two-photon induced, dual-channel fluorescence imaging.

On page 2, paragraph 1 of the Office Action of July 13, 2007, the Examiner objects to the disclosure for informalities: On page 7, at line 12, "fluorine" should read – fluorene--; on page 6 at lines 11-12, the application number should be replaced by the U.S. Patent number; these informalities have been addressed and corrected. It is respectfully requested that the objections to the disclosure be withdrawn.

Paragraphs 2 and 3 of the Office Action of July 13, 2007 are related to the rejection of Claim 6 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the invention because of a spelling error wherein "fluorine" should read – fluorene--; Claim 6 has been amended to correct the error, accordingly, Applicant request the withdrawal of the rejection of Claim 6 under 335 U.S.C. 112, second paragraph.

Paragraphs 4 and 5 of the Office Action of July 13, 2007 set forth the statutory basis for rejecting claims under 35 U.S.C. 102 and 35 U.S.C. 103(a).

Paragraph 6 of the Office Action of July 13, 2007 states that Claims 1 and 3 are rejected under 35 U.S.C. 102(b) as being fully anticipated by Fleming et al. WO 01/96959. The Examiner argues that "Fleming et al. teaches … a photosensitive composition …(a photo-acid generator) and … (a two photon dye) coated to a thickness of 60 microns written upon using a Ti:sapphire operating at 800 nm…" The use of fluorene dyes is disclosed on page 28." Applicant's amended independent claims now require the use of the photo-acid generator to undergo two-photon absorption to generate a proton ion (H^+) or superacid that reacts with the fluorene dye for image writing. Thus, Applicant does not use light in writing to change optical properties of the storage medium, whereas, light is used in Fleming et al. to change the optical properties of a storage medium to create a holographic image.

One major distinction between Applicant's invention and the disclosure by Fleming et al is: Fleming et al. uses fluorene dyes that are exposed to light to store data; in contrast, Applicant uses fluorene dyes to read data in a fluorescent based readout.

It is further noted that on pages 30 and 31 of Fleming et al. fluorene dyes that were first synthesized and published by Applicant are disclosed without acknowledging the contributions from Applicant's publications. See enclosed publications (*Attachment A*) : K. Belfield, et al, "New Two-Photon Absorbing Fluorene Derivatives: Synthesis and Nonlinear Optical Characterization" in Organic Letters Vol. 1 (10) pages 1575-1578 (1999); K. Belfield, et al "Synthesis of New Two-Photon Absorbing Fluorene Derivatives via Cu-Mediated Ullmann Condensations," The Journal of Organic Chemistry Vol. 65 (15) pages 4475-4481 July 28, 2000; K. Belfield, et al "Multiphoton-absorbing organic materials for microfabrication, emerging optical applications and non-destructive three-dimensional imaging," Journal of Physical Organic Chemistry Vol. 13, pages 837-849 (2000).

Another major distinction between Applicant's invention and the disclosure by Fleming et al. is the use of a photoreactive species including monomers, oligomers, and cross-linkable polymers wherein light is used to create polymeric material or cross-linked material with holographic elements; in contrast, Applicant uses a transparent polymeric material and DOES NOT initiate polymerization reactions or the like.

Fleming et al. teach a distinctly different technology and use of fluorene compounds and photo-acid generators. Applicant uses a photo-acid generator that undergoes two-photon absorption, generating a super acid (or proton ion); the acid then reacts with the fluorene derivative to change the optical properties of the medium for storage. In contrast to Applicant's use, Fleming et al use fluorene derivatives as a photosensitizer to make another

photoreactive material (e.g., monomer, oligomer, and the like) more sensitive to two-photon absorption. Applicant DOES NOT use fluorene dye as a photosensitizer.

Applicant does use fluorene compounds in the readout of stored data by exciting the fluorene derivatives and detecting the changes in optical properties of the medium; but the fluorene derivatives are not undergoing any photochemical reactions. They are simply there for the readout.

Thus, Fleming et al is teaching a technology related to holographic data storage and preparation of complex, three-dimensional objects, while Applicant is teaching a chemical process yielding image formation for compact disc (CD) and Digital Versatile Disc (DVD) and related technology using a photosensitive polymeric film containing a photo-acid generator and an acid-sensitive reactive dye (fluorophore), which allows two-photon induced, dual-channel fluorescence imaging.

In addition to the above arguments regarding the distinctions between Applicant's invention and the teachings of Fleming et al., Applicants have submitted a Declaration under 37 C.F.R. § 1.131 which antedates the Fleming et al reference. See *Attachment B* with Exhibits A and B.

In view of the arguments and Declarations above, Applicant respectfully requests the withdrawal of the rejection of Claims 1 and 3 under 35 U.S.C. 102(b) as being fully anticipated by Fleming et al. WO 01/96959.

On page 3 of the Office Action of July 13, 2007, Applicant acknowledges the paragraphs numbered 7 – 11 as being left blank.

Paragraph 12 on page 3 of the Office Action of July 13, 2007, Claims 1-7, 9, 10 and 15-17 are rejected under 35 U.S.C. 102(b) as being fully anticipated by Belfield et al. "Three

dimensional two photon imaging in polymeric materials" Proc. SPIE Vol. 4459 pp. 281-289

(01/2002) or the corresponding presentation (July 2001).

Applicant has amended the specification to identify this application as <u>a continuation-</u>

<u>in-part of co-pending U.S. Patent Application SN 10/306,960 filed November 27, 2002, now</u>

<u>U.S. Patent 7,001,708 that issued on February 21, 2006, which claims the benefit of priority</u>

<u>based on U.S. Provisional application No. 60/339,283 filed December 11, 2001 and U.S.</u>

<u>Provisional application No. 60/333,972 filed on November 28, 2001, and</u> this invention

<u>further</u> claims <u>the benefit of</u> priority based on United States Provisional Application No.

60/463,426 filed April 16, 2003.

Applicant notes that the subject application was filed on April 09, 2004 which makes

it co-pending with commonly owned <u>SN 10/306,960 filed on November 27, 2002</u> and now U.

S. Patent 7,001,708 that issued on February 21, 2006. Further, the subject application

acknowledges the relationship of the instant invention to that disclosed in the now issued U.S.

Patent 7,001,708 on page 6 of the specification lines 9 – 12.

Applicant disagrees with the Examiner that the disclosure of a binary encoding

scheme would fully anticipate the use of a ternary encoding scheme for a WORM data

storage medium with two-photon fluorescence readout to increase data storage capacity by

50%. However, to advance the prosecution of the subject application, Applicant has filed a

Petition under 37 CFR 1.78(a)(3) and 1.78(a)(6) for an Unintentionally Delayed Domestic

Priority Claim to make the present application a continuation-in-part of a commonly owned,

earlier issued U.S. Patent 7,001,708. With the amendment of the domestic priority claim for

the subject application, a copy of which is enclosed as *Attachment C*, Applicant respectfully

requests the withdrawal of the rejection of Claims 1-7, 9, 10 and 15-17 are rejected under 35

U.S.C. 102(b) as being fully anticipated by Belfield et al., Proc. SPIE Vol. 4459 pp. 281-289 (01/2002) or the corresponding presentation (July 2001).

In paragraph 13 on page 4 of the Office Action of July 13, 2007, Claims 1 – 22 are rejected under 35 U.S. C. 103(a) as being unpatentable over Belfield et al. "Three dimensional two photon imaging in polymeric materials" Proc. SPIE Vol. 4459 pp. 281-289 (01/2002) or the corresponding presentation (July 2001), in view of Glushko et al. '671 and Fourkas et al. '063.

The Examiner has made an argument that essentially states, "it would be obvious to" discover Applicants invention by reading the teachings of Belfield et al. Proc. SPIE *supra*, in view of Glushko et al '671 teachings regarding fluorescent 3-D optical memory devices and Fourkas et al '063 that teaches an apparatus and materials for 3-D optical data storage and retrieval that includes a Ti:Sapphire laser in a scanner. Applicant disagrees and has presented evidence that Applicant has used a chemical process that yields image formation within a photosensitive polymeric film containing a photoacid generator and an acid-sensitive reactive dye (fluorophore), which allows two-photon induced, dual-channel fluorescence imaging. But for the Belfield et al. Proc. SPIE *supra* reference, Glushko et al '671 and Fourkas et al '063 are only state of the art references for 3-D optical recording and not relevant to Applicant's use of a photo-acid generator and a reactive dye in a WORM optical data storage system or device.

Further, with the amendment of Applicant's domestic priority claim for the subject application the domestic priority claim for the subject application to make this application a continuation-in-part of a commonly owned, earlier issued U.S. Patent 7,001,708, a copy of which is enclosed as *Attachment C*, Applicant respectfully requests the withdrawal of the

rejection of Claims 1 – 22 under 35 U.S. C. 103(a) as being unpatentable over Belfield et al.

"Three dimensional two photon imaging in polymeric materials" Proc. SPIE Vol. 4459 pp.

281-289 (01/2002) or the corresponding presentation (July 2001), in view of Glushko et al.

'671 and Fourkas et al. '063.

In paragraph 14 on page 5 of the Office Action of July 13, 2007, Claims 1-22 are

rejected under 35 U.S.C. 103(a) as being unpatentable over over Belfield et al. "Three

dimensional two photon imaging in polymeric materials" Proc. SPIE Vol. 4459 pp. 281-289

(01/2002) or the corresponding presentation (July 2001), in view of Glushko et al. '671 and

Fourkas et al. '063, further in view of Rentzepis et al. '031 and Tanaka et al. Appl. Phys.

Lett., Vol. 80 (2) pp. 312-314 (01/2002).

Applicant maintains the arguments stated above with regard to overcoming the

rejections in paragraph 13 on page 5 of the Office Action of July 13, 2007. The additional

references Rentzepis et al. '031 and Tanaka et al. Appl. Phys. Lett., _supra_ do not cure the

invalid rejection.

Applicant acknowledges the work of Rentzepis et al using spiropyran, in the

background discussion of the present invention. Applicant notes and distinguishes the

present invention as using a chemical process yielding image formation of a stable and long-

term nature, in contrast to Rentzepis et al. using materials known to undergo photobleaching

and photodegradation on prolonged exposure, hence not suitable for long term use. (See

page 2, lines 19-25 of the subject disclosure.)

Tanaka et al., _supra_ use laser light for the formation of submicron features; whereas,

Applicant uses a chemical process for image formation, as now claimed. Applicant's

amended claims are now distinguished from all the technology discussed in references cited by the Examiner that use laser light for recording and storage of data in polymeric medium.

Further, with the amendment of Applicant's domestic priority claim for the subject application to make this application a continuation-in-part of a commonly owned, earlier issued U.S. Patent 7,001,708, a copy of which is enclosed as *Attachment C*, Applicant respectfully requests the withdrawal of the rejection of Claims 1 – 22 under 35 U.S. C. 103(a) as being unpatentable over Belfield et al. "Three dimensional two photon imaging in polymeric materials" Proc. SPIE Vol. 4459 pp. 281-289 (01/2002) or the corresponding presentation (July 2001), in view of Glushko et al. '671 and Fourkas et al. '063, further in view of Rentzepis et al. '031 and Tanaka et al. Appl. Phys. Lett., Vol. 80 (2) pp. 312-314 (01/2002).

There must be a reason apparent at the time the invention was made to the person of ordinary skill in the art for applying the teaching at hand, or use of the teaching as evidence of obviousness will entail prohibited hindsight." *In re Nomiya,* 184 USPQ 607 (CCPA, 1975).

Paragraph 15 on page 6 of the Office Action of July 13, 2007 discusses the judicially created doctrine of nonstatutory double patenting which is then applied in the rejections set forth by the Examiner in Paragraphs 16, 17, 18 and 19.

In paragraph 16 on page 6 of the Office Action of July 13, 2007, Claims 3-22 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-4 of U.S. Patent No. 7,001,708, in view of Belfield, et al. "Three dimensional two photon imaging in polymeric materials" Proc. SPIE vol. 4459 pp. 281-289 (01/2002) or the corresponding presentation (July 2001), in view of Glushko, et al. and Fourkas, et al.

The Examiner argues that "it would have been obvious to one skilled in the art to [modify] the invention of claims 1-4 of U.S. Patent No. 7,001,708 by forming multiple recording layers ...[up to 100 layers] with spacers based upon the teach[ings] of Glushko et al.'671 and Fourkas et al. as each layer will increase the information density of the medium with a reasonable expectation of success given the exemplification of two layers by Belfield et al. Proc. SPIE..." Applicant disagrees. It was unexpected that instead of using a binary encoding scheme as taught in Belfield's U.S. Patent '708, Applicant could use a ternary encoding scheme and increase the data storage capacity by 50%. The use of a ternary encoding scheme is not taught, suggested or discussed by Belfield '708, Belfield, Proc. SPIE, supra, Glushko et al. '671 or Fourkas et al.

Further, Applicant has amended the domestic priority claim for the subject application to make this application a continuation-in-part of a commonly owned, earlier issued U.S. Patent 7,001,708, a copy of which is enclosed as *Attachment C*, therefore, Applicant respectfully requests the withdrawal of the rejection of Claims 3-22 on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-4 of U.S. Patent No. 7,001,708, in view of Belfield, et al. "Three dimensional two photon imaging in polymeric materials" Proc. SPIE vol. 4459 pp. 281-289 (01/2002) or the corresponding presentation (July 2001), in view of Glushko, et al. and Fourkas, et al.

In paragraph 17 on page 7 of the Office Action of July 13, 2007, Claims 1-22 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-15 and 17-21 of copending Application No. 11/256552, in view of Belfield, et al. "Three dimensional two photon imaging in polymeric materials" Proc.

SPIE vol. 4459 pp. 281-289 (01/2002) or the corresponding presentation (July 2001), in view

of Glushko, et al. and Fourkas, et al. '063

Applicant notes that the Examiner states "this is a provisional obviousness-type

double patenting rejection. However, in view of arguments given above and the amendment

of the domestic priority claim for the subject application to make this application a

continuation-in-part of a commonly owned, earlier issued U.S. Patent 7,001,708, a copy of

which is enclosed as *Attachment C*, therefore, Applicant respectfully requests the withdrawal

of the rejection of Claims 3-22 on the ground of nonstatutory obviousness-type double

patenting as being unpatentable over claims 1-15 and 17-21 of copending Application No.

11/256552, in view of Belfield, et al. "Three dimensional two photon imaging in polymeric

materials" Proc. SPIE vol. 4459 pp. 281-289 (01/2002) or the corresponding presentation

(July 2001), in view of Glushko, et al. and Fourkas, et al.

In paragraph 18 on page 8 of the Office Action of July 13, 2007, Claims 3-22 are

provisionally rejected on the ground of nonstatutory obviousness-type double patenting as

being unpatentable over claims 1-15 and 17-21 of copending Application No. 11/272189, in

view of Belfield, et al. "Three dimensional two photon imaging in polymeric materials" Proc.

SPIE vol. 4459 pp. 281-289 (01/2002) or the corresponding presentation (July 2001), in view

of Glushko, et al. '671 and Fourkas, et al. '063.

Again the Examiner argues "it would have been obvious to one skilled in the art to

[modify] the invention of copending Application No. 11/256552 by forming multiple

recording layers ...[up to 100 layers] with spacers based upon the teach[ings] of Glushko et

al.'671 and Fourkas et al. as each layer will increase the information density of the medium

with a reasonable expectation of success given the exemplification of two layers by Belfield

et al. Proc. SPIE..." Applicant continues to disagree. The use of Applicant's ternary encoding scheme instead of a binary encoding scheme to increase optical data storage by 50% is not taught, suggested or discussed by Belfield in copending Application No. 11/256662, Belfield, Proc. SPIE, *supra*, Glushko et al. '671 or Fourkas et al. '063.

Further, Applicant has amended the domestic priority claim for the subject application to make this application a continuation-in-part of a commonly owned, earlier issued U.S. Patent 7,001,708, a copy of which is enclosed as *Attachment C*; therefore, Applicant respectfully requests the withdrawal of the provisional rejection of Claims 3-22 on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-15 and 17-21 of copending Application No. 11/272189, in view of Belfield, et al. "Three dimensional two photon imaging in polymeric materials" Proc. SPIE vol. 4459 pp. 281-289 (01/2002) or the corresponding presentation (July 2001), in view of Glushko, et al. '671 and Fourkas, et al. '063.

In paragraph 19 on page 8 of the Office Action of July 13, 2007, Claims 3-22 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 29-30 of copending Application No. 11/707553, in view of Belfield, et al. "Three dimensional two photon imaging in polymeric materials" Proc. SPIE vol. 4459 pp. 281-289 (01/2002) or the corresponding presentation (July 2001), in view of Glushko, et al. '671 and Fourkas, et al. '063.

The Examiner makes the same argument that it would have been obvious to form multiple recording layers based on the teachings in the cited art. The invention is not solely based on multiple layers of material for recording and reading data; but on a novel method that uses a chemical process to record and store data and the use of a ternary coding scheme

to increases the storage capacity by 50% over that of a binary encoding scheme, then a two-photon fluorescent readout of the stored data using a fluorene dye.

The use of Applicant's ternary encoding scheme instead of a binary encoding scheme to increase optical data storage by 50% is not taught, suggested or discussed by Belfield in copending Application No. 11/707553, Belfield, Proc. SPIE, *supra*, Glushko et al. '671 or Fourkas et al. '063.

Further, Applicant has amended the domestic priority claim for the subject application to make this application a continuation-in-part of a commonly owned, earlier issued U.S. Patent 7,001,708, a copy of which is enclosed as *Attachment C*; therefore, Applicant respectfully requests the withdrawal of the provisional rejection of Claims 3-22 on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 29-30 of copending Application No. 11/707553, in view of Belfield, et al. "Three dimensional two photon imaging in polymeric materials" Proc. SPIE vol. 4459 pp. 281-289 (01/2002) or the corresponding presentation (July 2001), in view of Glushko, et al. '671 and Fourkas, et al. '063.

Applicant acknowledges the prior art made of record in paragraph 20 on page 9 of the Office Action of July 13, 2007 (Cumpston et al '228 and Hesselink et al '148) and not relied upon.

In view of Applicant's arguments, amendments to the specification and claims, the application and Claims 1-22 are believed in condition for allowance; allowance is respectfully requested.

If the Examiner believes that an interview would be helpful, the Examiner is requested to contact the attorney at the below listed number.

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Respectfully submitted,

Date: _____ 10/18/07
Customer No.: 23717

_____
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ATTACHMENT A

References included in this Attachment:

1. <u>**Organic Letters**</u> **Vol. 1, No. 10 (1999) pages 1575-1578**

2. <u>**The Journal of Organic Chemistry**</u> **Vol. 65 (15) July 28, 2000 pages 4475 – 4481**

3. <u>**Journal of Physical Organic Chemistry**</u> **Vol. 13 (2000) pages 837-849**

New Two-Photon Absorbing Fluorene Derivatives: Synthesis and Nonlinear Optical Characterization

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ABSTRACT

R = P(O)(OCH₂CH₃)₂ (2), NO₂ (3)

R = $P(O)(OCH_2CH_3)_2$ (2), NO_2 (3)

Efficient Pd-catalyzed Heck coupling methodology was employed to provide two new fluorene derivatives with phosphonate (2) and nitro (3) electron-withdrawing functionalities. Both derivatives exhibit two-photon absorption (2PA), as determined by nonlinear absorption measurements using a femtosecond pump/white light continuum probe "NLO spectrometer". Both fluorene derivatives have high 2PA cross sections (650 and 1300×10^{-50} cm^4 s photon^{-1} molecule^{-1} for compounds 2 and 3, respectively).

The quest for organic materials exhibiting high nonlinear optical (NLO) absorptivities has increased dramatically over the past several years.[1] One nonlinear absorption process, two-photon absorption (2PA), is the subject of fast-growing interest in the chemistry, photonics, and biological imaging communities. Several current and emerging technologies exploit the two-photon absorption phenomenon, including optical power limiting materials,[2] two-photon fluorescence imaging,[3] two-photon photodynamic cancer therapy,[4] and two-photon microfabrication.[5] The 2PA process considered here involves the simultaneous absorption of two photons, either degenerate or nondegenerate, at wavelengths well beyond the linear absorption spectrum of a particular molecule.[6] Though a subject of contemporary investigation, the simultaneous absorption of two quanta of energy was first predicted in 1931 by Goeppert-Mayer.[7] We report, herein, the synthesis and nonlinear optical characterization of two specific fluorene derivatives that exhibit relatively large 2PA cross sections, a measure of two-photon absorptivity. Nondegenerate 2PA spectra were recorded with our recently developed "NLO spectrometer".[8]

Why are two-photon absorbing materials so intensely pursued? Let us consider a major feature that distinguishes single-photon absorption (1PA) from two-photon absorption (2PA): the rate of energy (light) absorption as a function of incident intensity. In single-photon absorption, the rate of light absorption is directly proportional to the incident

(1) Kershaw, S. In *Characterization Techniques and Tabulations for Organic Nonlinear Optical Materials*; Kuzyk, M. G., Dirk, C. W., Eds.; Marcel Dekker: New York, 1998, Chapter 7.

(2) Tutt, L, W.; Boggess, T. F. *Prog. Quantum Electr.* **1993**, *17*, 299.

(3) Denk, W.; Strickler, J. H.; Webb, W. W. *Science* **1990**, *248*, 73.

(4) Wachter, E. A.; Partridge, W. P.; Fisher, W. G.; Dees, H. C.; Petersen, M. G. *Proc. SPIE—Int. Soc. Opt. Eng.* **1998**, *3269*, 68.

(5) See, for example: Belfield, K. D.; Ren, X.; Hagan, D. J.; Van Stryland, E. W.; Dubikovsky, V.; Miesak, E. J. *Polym. Mater. Sci. Eng.* **1999**, *81*, 79.

(6) Birge, R. R. In *Ultrasensitive Laser Spectroscopy*; Kliger, D. S., Ed.; Academic Press: New York, 1983, Chapter 2.
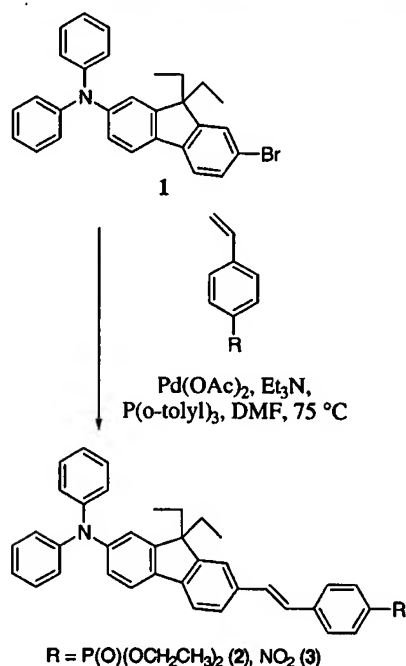
(7) Goeppert-Mayer, M. *Ann. Phys.* **1931**, *9*, 273.

(8) Negres, R. A.; Van Stryland, E. W.; Hagan, D. J.; Belfield, K. D.; Schafer, K. J.; Przhonska, O. V.; Reinhardt, B. A. *Proc. SPIE—Int. Soc. Opt. Eng.* **1999**, *3796*, in press.

intensity ($dw/dt \propto I$), i.e., as the incident intensity is increased, the rate of photon absorption increases linearly for the molecule in question. By contrast, in simultaneous two-photon absorption, the rate of energy absorption is proportional to the square of the incident intensity ($dw/dt \propto I^2$).[1,6] This quadratic, or nonlinear, dependence has substantial implications. For example, in a medium containing one-photon absorbing chromophores, significant absorption occurs all along the path of a focused beam of suitable wavelength light. This can lead to, e.g., photodegradation or photobleaching. In 2PA, negligible absorption occurs except in the immediate vicinity of the focal point of a light beam of appropriate energy. This allows spatial resolution along the beam axis as well as radially and is the principal basis for two-photon fluorescence imaging.[3] The simultaneous absorption of two or more photons requires high peak power, which is now available from commercially available ultrafast pulsed lasers. Thus, certain materials can undergo nonresonant 2PA at wavelengths far beyond their linear absorption spectrum.

Two fluorene derivatives with different electron-withdrawing groups were synthesized via Heck coupling reactions, as illustrated in Scheme 1. The fluorenyl ring system was

Near-quantitative Pd-catalyzed Heck coupling[9] of 2-bromo-7-N,N-diphenylamino-9,9-diethylfluorene **1** (prepared in three steps from fluorene via dibromination, diethylation, and Ullmann-type coupling with diphenylamine as previously reported[10]) with either 4-vinylbenzene phosphonic acid diethyl ester or 4-nitrostyrene afforded novel fluorene dyes **2** and **3**, respectively. 4-Vinylbenzene phosphonic acid diethyl ester and 4-nitrostyrene were prepared from 4-bromo-acetophenone and 4-nitroacetophenone, respectively, by established procedures.[11] The Heck coupling reactions were conducted with $Pd(OAc)_2$, tri-o-tolylphosphine, and Et_3N as base in DMF at 75 °C for 15 h. Phosphorylated fluorene derivative **2** was isolated in 92% yield as a fluorescent yellow solid and fully characterized.[12] The UV—visible absorption spectrum of **2** in CH_3CN extended out to about 480 nm with two λ_{max}, one at 308 nm and the other at 383 nm.

Nitro-containing fluorene derivative **3** was obtained in 90% yield as a fluorescent orange-red solid and fully characterized.[13] The visible absorption of **3** in CH_3CN extended out to about 550 nm with two λ_{max}, one at 309 nm and the other at 414 nm.

Linear and nonlinear absorption (nondegenerate 2PA) spectra for fluorene derivatives **2** and **3** are shown in Figures 1 and 2, respectively.
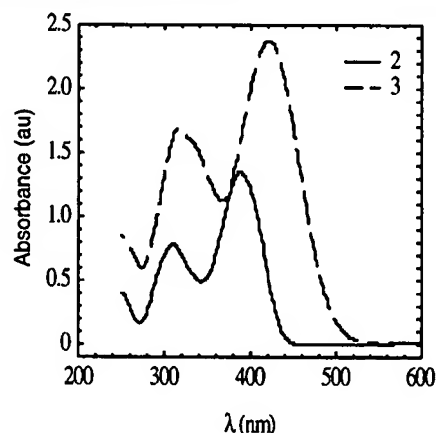
Scheme 1

R = P(O)(OCH₂CH₃)₂ (2), NO₂ (3)

Figure 1. Linear absorption spectra (absorption units vs wavelength) for compounds **2** and **3**.

chosen to serve as a thermally and photochemically stable π-conjugated system that can be readily functionalized in the 2-, 7-, and/or 9-positions. Such functionalization facilitates the systematic preparation of derivatives with varying electronic character for molecular structure/nonlinear absorption relationships. Polarizable molecular structures with relatively long conjugation lengths may lead to large two-photon absorptivities.[1] In the current work, derivatives bearing electron-withdrawing groups (phosphonate and nitro) of different strengths were prepared and studied.

Details of the nonlinear absorption measurements and the "NLO spectrometer" have been described elsewhere.[8] Briefly, femtosecond pump probe experiments were performed in which the pump beam wavelength was selected to be at a low enough energy (long wavelength, 1210 nm) to prevent degenerate 2PA. The probe beam consisted of a femtosecond white light continuum (WLC) generated by irradiation of a sapphire window.

(9) Belfield, K. D.; Chinna, C.; Schafer, K. J. *Tetrahedron Lett.* **1997**, *38*, 6131.

(10) Reinhardt, B. A.; Brott, L. L.; Clarson, S. J.; Dillard, A. G.; Bhatt, J. C.; Kannan, R.; Yuan, L.; He, G. S.; Prasad, P. N. *Chem. Mater.* **1998**, *10*, 1863.

(11) Belfield, K. D.; Chinna, C.; Najjar, O. *Macromolecules* **1997**, *31*, 22918.

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The 2PA spectra illustrated in Figure 2 show the 2PA cross section (in units of cm^4 s photon^{-1} molecule^{-1}). Fluorene **2**
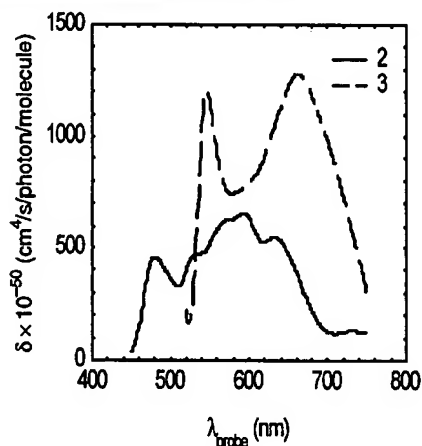

Figure 2. Two-photon absorption spectra [2PA cross section, δ (cm^4 s photon^{-1} molecule^{-1}), vs probe wavelength] for compounds **2** and **3** using a 1210 nm pump beam.

exhibits a maximum 2PA cross section of ca. 650×10^{-50} cm^4 s photon^{-1} molecule^{-1} at WLC wavelength 605 nm. The 2PA cross section of fluorene **3** is significantly higher, ca. 1300×10^{-50} cm^4 s photon^{-1} molecule^{-1} at WLC wavelength 670 nm, and comparable to very large 2PA cross sections that were recently reported for another class of organic compounds (on the order of 500 to 1100×10^{-50} cm^4 s photon^{-1} molecule^{-1}).[14]

Figures 3 and 4 display the linear and 2PA spectra of derivatives **2** and **3**, respectively, plotted as absorption vs

Figure 3. Linear and nonlinear absorption spectra of **2** (absorption units vs eV).

total photon energy in eV (the linear wavelengths were converted to photon energy while the wavelengths of the pump and probe beams were converted to photon energy

and summed for the 2PA spectra). Relatively good correlation between the linear and nonlinear spectra was observed. This is to be expected for molecules having an extended π-conjugated system and permanent dipole moment. For these systems, the parity can mix such that the spectra of one- and two-photon allowed states overlap. Further overlap of the one- and two-photon spectra arise from the near degeneracy of one- and two-photon allowed states and the vibronic structure of the molecules.[6]
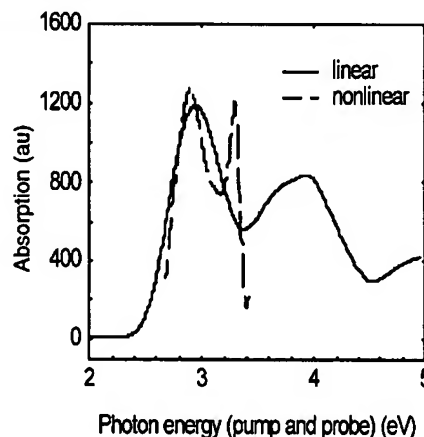

Figure 4. Linear and nonlinear absorption spectra of **3** (absorption units vs eV).

Thus, efficient Pd-catalyzed Heck coupling methodology afforded two new fluorene derivatives with phosphonate (**2**) and nitro (**3**) electron-withdrawing functionalities in at least 90% yield. Both derivatives exhibit two-photon absorption (2PA), as determined by nonlinear absorption measurements using a femtosecond pump/white light continuum probe "NLO spectrometer". The large 2PA cross sections for the two fluorene derivatives (650 and 1300×10^{-50} cm^4 s photon^{-1} molecule^{-1} for compounds **2** and **3**, respectively) open the door for potential applications of these chromophores in optical power limiting and multiphoton fluorescence imaging applications.

(12) Characterization data for compound **2**: MS (EI) m/z 627 (M$^+$), 541 (M − 2(C$_2$H$_5$)), 168 (C$_{12}$H$_{10}$N$^+$), 77 (C$_6$H$_5$$^+$); UV−vis (CH$_3$CN) λ_{max} = 308 and 383 nm; mp = 185−186 °C. Anal. Calcd: C = 78.45; H = 6.74; N = 2.23. Found: C = 78.22; H = 6.94; N = 1.85. ^{1}H NMR (200 MHz, CDCl$_3$) δ: 7.85−6.98 (m, 20H, ArH), 7.12 (q, J = 7.4 Hz, 2H, trans-CH=CH), 4.14 (q, J = 7.0 Hz, 4H, OCH$_2$), 1.95 (m, J = 7.1 Hz, 4H, CH$_2$), 1.35 (t, J = 7.0 Hz, 6H, ester CH$_3$), 0.40 (t, J = 7.1 Hz, 6H, CH$_3$).
(13) Characterization data for compound **3**: MS (EI) m/z 536 (M$^+$), 490 (M − NO$_2$), 168 (C$_{12}$H$_{10}$N$^+$), 77 (C$_6$H$_5$$^+$); UV−vis (CH$_3$CN) λ_{maxima} = 309 and 414 nm; mp = 192−194 °C. Anal. Calcd: C = 82.81; H = 6.01; N = 5.22. Found: C = 82.75; H = 5.89; N = 4.90. ^{1}H NMR (200 MHz, CDCl$_3$) δ: 8.17 (d, J = 9.3 Hz, 2H, ArH ortho to NO$_2$), 7.60−6.93 (m, 18H, ArH), 7.05 (q, J = 8.0 Hz, 2H, trans-CH=CH), 1.88 (m, J = 7.0 Hz, 4H, CH$_2$), 0.30 (t, J = 7.0 Hz, 6H, CH$_3$).
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Articles

Synthesis of New Two-Photon Absorbing Fluorene Derivatives via Cu-Mediated Ullmann Condensations

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The Ullmann amination reaction was utilized to provide access to a number of fluorene analogues from common intermediates, via facile functionalization at positions 2, 7, and 9 of the fluorene ring. Through variation of amine or iodofluorene derivative, analogues bearing substitutents with varying electron-donating and electron-withdrawing ability, e.g., diphenylamino, bis-(4-methoxyphenyl)amine, nitro, and benzothiazole, were synthesized in good yield. The novel fluorene derivatives were fully characterized, including absorption and emission spectra. Didecylation at the 9-position afforded remarkably soluble derivatives. Target compounds 4, 5, and 9 are potentially useful as fluorophores in two-photon fluorescence microscopy. Their UV–vis spectra display desirable absorption in the range of interest suitable for two-photon excitation by near-IR femtosecond lasers. Preliminary measurements of two-photon absorption indicate the derivatives exhibit high two-photon absorptivity, affirming their potential as two-photon fluorophores. For example, using a 1210 nm femtosecond pump beam, diphenylaminobenzothiazolylfluorene 4 exhibited nondegenerate two-photon absorption, with two-photon absorptivity (δ) of ca. 820×10^{-50} cm^4 s $photon^{-1}$ $molecule^{-1}$ at the femtosecond white light continuum probe wavelength of 615 nm.

Introduction

Organic materials exhibiting significant nonlinear optical effects have been a subject of intense investigation recently.[2] In particular, compounds that undergo strong nonlinear, multiphoton absorption are being investigated as materials for a wide variety of potential applications in areas ranging from optical sciences, biophotonics, materials sciences, and photochemistry, to name a few.

Multiphoton absorption can be defined as simultaneous absorption of two or more photons through virtual states in a medium.[3] More specifically, the nonlinear process of two-photon absorption (TPA) has been gaining greater interest among these multidisciplinary areas, particularly in the rapidly developing field of multiphoton fluorescence imaging.[4] Since the probability of a TPA process is proportional to the square of the intensity, photoexcitation and upconverted fluorescence is confined to the focal volume. Many other applications of TPA have been suggested, ranging from their use as three-dimen-

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sional optical data storage devices, optical switching devices for optical computing and telecommunications applications, as dyes in laser technology, optical storage devices, and photodynamic cancer therapy.[5-9] Recently, we reported two-photon induced polymerization as a means of three-dimensional microfabrication.[10]

The theory of the simultaneous absorption of two photons was developed by Goeppert-Mayer in 1931,[3] but remained mainly a conceptual curiosity until the advent of the pulsed laser providing very high-intensity light. For simplicity, two-photon absorption can be conceptualized from a semiclassical perspective.[11] In the TPA process, molecules exposed to high intensity light can undergo near simultaneous absorption of two photons mediated by a so-called "virtual state", a state with no classical analogue. The combined energy of the two photons accesses a stable excited state of the molecule. As light passes through a molecule, the virtual state may form, persisting for a very short duration (on the order of a few femtoseconds). TPA can result if a second photon arrives before decay of this virtual state, with the probability of TPA scaling with the square of the light intensity. This process is generally termed simultaneous two-photon absorption. Two-photon absorption, thus, involves the *concerted* interaction of both photons that combine their energies to produce an electronic excitation analogous to that conventionally caused by a single photon of a correspondingly shorter wavelength. Unlike single photon absorption, whose probability is linearly proportional to the incident intensity, the TPA process depends on both a spatial and temporal overlap of the incident photons and takes on a quadratic (nonlinear) dependency on the incident intensity.

For nonpolar molecules with a low-lying, strongly absorbing state near the virtual level, only excited states that are forbidden by single-photon selection rules can be populated via two-photon absorption.[11] The probability that this low-lying state can contribute to the virtual state is predicted by Heisenberg's uncertainty principle, with a virtual state lifetime approximated as $h/(4\pi\Delta E)$, where h is Planck's constant and ΔE is the energy difference between the virtual and actual states. In contrast, strong TPA can occur in polar molecules by a different mechanism in which a large change in dipole moment ($\Delta\mu > 10$ D) occurs upon excitation of the ground to an excited state. Single-photon allowed states can then be accessed via TPA, and the virtual state lifetime is proportional to $\Delta\mu^2$. In this case, both the ground and excited states can participate in formation of the virtual state, enhancing TPA. Two-photon absorptivity, δ, is expressed in Goppert-Mayers units (GM), with 1 GM = 1×10^{-50} cm^4 s molecule^{-1} photon^{-1}. In polar molecules

with large $\Delta\mu$ between the ground and excited states, δ values in excess of 10 GM have been observed.[11]

Particular molecules can undergo upconverted fluorescence through nonresonant two-photon absorption using near-IR radiation, resulting in an energy emission greater than that of the individual photons involved (upconversion). The use of a longer wavelength excitation source for fluorescence emission affords advantages not feasible using conventional UV or visible fluorescence techniques, e.g., deeper penetration of the excitation beam and reduction of photobleaching.[4] Ar ion (488 nm) and frequency-doubled Nd:YAG (532 nm) lasers are the commonly used light sources for conventional (single-photon) laser scanning confocal microscopy (due to their ready availability and low cost). Such light sources require fluorophores with strong absorbance near these wavelengths. Two-photon laser scanning fluorescence microscopy systems, on the other hand, are generally configured with a Ti:sapphire laser with 80–120 fs pulse output in the near-IR region (700–900 nm). Consequently, many typical single-photon fluorophores undergo only weak TPA in this region, since a fluorophore with λ_{max} of 490 nm would be expected to undergo two-photon absorption at ca. 980 nm (a wavelength where the output power of commercial Ti:sapphire lasers is practically too low to be useful). Thus, commercial fluorophores are far from being optimized for use in two-photon fluorescence microscopy. A more reasonable absorption maximum for such chromophores is 380–420 nm (facilitating use of near-IR femtosecond sources in the range of 760–840 nm), since the TPA λ_{max} will be approximately twice the wavelength of the single-photon λ_{max}.

Although numerous fluorophores for conventional fluorescence microscopy are commercially available, few efforts have addressed the need for multiphoton absorbing fluorophores. As part of a program to establish an empirical body of nonlinear absorptivity as a function of molecular structure, and prepare efficient two-photon absorbing fluorophores, we are preparing and characterizing a series of compounds with systematic variation in molecular structure. Polar organic compounds such as 4-nitroaniline and 4-amino-4′nitrobiphenyl undergo increases in dipole moments on excitation from 6 to 14 D and from 6 to 20 D, respectively.[12] Thus, such compounds are expected to possess high two-photon absorptivity, as discussed above. We chose the fluorenyl ring system to serve as a thermally and photochemically stable π-conjugated analogue of the 4,4′-disubstituted biphenyl derivatives. Locking the biphenyl unit into the fluorenyl ring provides greater electron delocatization through increased π molecular orbital overlap between the rings, enhancing molecular polarizability. Importantly, fluorene can be readily functionalized in the 2, 7, and/or 9-positions. We recently reported the synthesis, linear optical, and nonlinear optical characterization of two fluorene derivatives, prepared by Pd-catalyzed Heck coupling, with high two-photon absorptivities.[13]

To prepare a series of derivatives for nonlinear absorption studies, we sought efficient preparation of key, common intermediates using synthetic methodology that would be readily adaptable for rapid functionalization.

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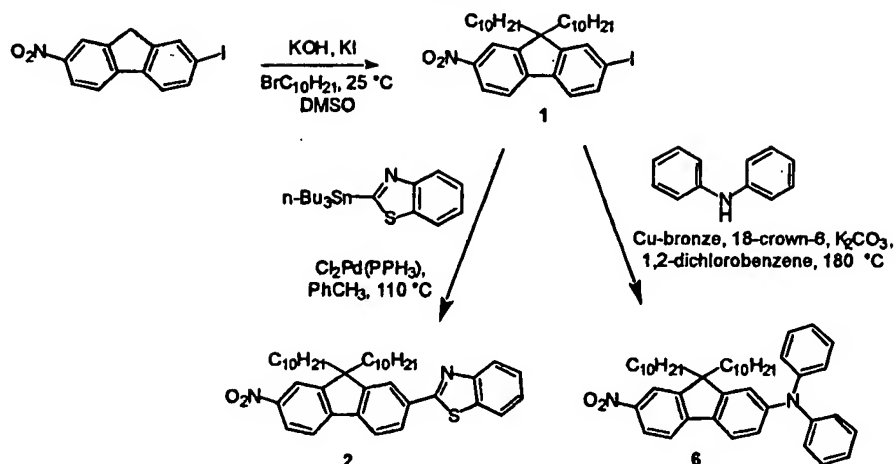(9) Maruo, S.; Nakamura, O.; Kawata, S. *Opt. Lett.* **1997,** *22,* 132.

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Scheme 1

We wish to report utilization of the Ullmann condensation reaction of arylamines with aryl iodides[14-16] to prepare a series of fluorene derivatives of varying electronic characteristics. Like 4-amino-4′nitrobiphenyl, such molecules should possess large transition dipoles and strong two-photon absorptivity. Structural confirmation data for these new derivatives are presented. Importantly, linear absorption and emission spectra and preliminary TPA data indicate that this class of compounds may be useful as two-photon fluorophores, providing new materials for imaging and other two-photon related applications.

Results and Discussion

The synthetic strategy employed involved the efficient preparation of key intermediates from fluorene. The fluorenyl ring system was chosen to suffice as a thermally and photochemically stable π-conjugated system, that can be readily functionalized on the 2, 7, and/or 9-positions. The electronic character of the π-conjugated central fluorene ring system was altered by varying the substitutents at the 2 and/or 7 positions. In addition, long alkyl groups at the 9-position were incorporated to enhance solubility. Details of the synthetic methodology and characterization data for the series of fluorene derivatives shown in Schemes 1–3 are discussed below.

2-Nitrofluorene was prepared in 80% yield by regiospecific nitration of fluorene with nitric acid in AcOH at 85 °C, following a literature preparation.[17] Regiospecific iodonation of 2-nitrofluorene with I_2, $NaNO_2$, AcOH, and H_2SO_4 at 115 °C afforded 7-iodo-2-nitrofluorene in 79% yield after recrystallization from AcOH (mp = 245–246 °C, lit.[18] mp 244–245 °C). CHN analysis was in good agreement with calculated values. Dialkylation of 7-iodo-2-nitrofluorene (Scheme 1) was accomplished by generation of the fluorenyl anion with KOH in DMSO and subsequent didecylation with 1-bromodecane in the presence of KI at room temperature.[19] 7-Iodo-9,9-didecyl-2-nitrofluorene (1) was obtained as a yellow solid in 77%

isolated yield after column chromatography. MS, ^1H, and ^{13}C NMR spectroscopic analysis confirmed formation of the desired product, with CHNI analysis in good agreement with calculated values. Evident in the ^1H NMR spectrum were characteristic resonances of the decyl groups, with the six terminal methyl protons appearing as a triplet centered at 0.83 ppm, broad multiplets at 0.56, 1.05, and 1.18 corresponding to 32 of the 36 methylene protons, and the four methylene protons α to the fluorenyl benzylic carbon (C9) at 1.99 ppm, a signature that will be common for the fluorene derivatives described hereafter. (2-Tri-n-butylstannyl)benzothiazole was prepared in 90% yield by treating benzothiazole with n-BuLi in THF at −75 °C, followed by addition of tri-n-butyltin chloride, as previously reported.[19] Pd-catalyzed Stille coupling was subsequently performed between 1 and (2-tri-n-butylstannyl)benzothiazole (Scheme 2) in refluxing toluene with either tetrakis(triphenylphosphine)palladium(0) or dichlorobis(triphenylphosphine)-palladium(II).[20] 2-(9,9-Didecyl-7-nitrofluoren-2-yl)benzothiazole (2) was obtained as yellow crystals in 61% isolated yield after column chromatography. Clearly evident in the FT-IR spectrum were absorptions attributable to the asymmetric and symmetric NO_2 stretching vibrations at 1519 and 1337 cm^{-1}, respectively. Also present was the benzothiazole C=N stretching vibration at 1589 cm^{-1}. Nitro derivative 2 exhibited UV−visible absorption ranging from 220 to 420 nm with λ_{max} at 365 nm. CHNS analysis results were in excellent agreement with calculated values. ^1H and ^{13}C NMR spectra were consistent, with the hallmark imine carbon of the benzothiazole ring (C2′) appearing downfield as expected at 168 ppm.[21]

Fast, quantitative reduction of 2 was achieved using hydrazine hydrate and 10% Pd/C in a 1:1 mixture of EtOH and THF at 70 °C (Scheme 2),[22] providing 7-benzothiazole-2-yl-9,9-didecylfluoren-2-ylamine (3) as a bright yellow viscous oil. Amine 3 was used directly in subsequent steps due to its oxidative lability. The UV−visible spectrum of 3 ranged from 210 to 450 nm, with the λ_{max} = 380 nm, exhibiting an expected 15 nm bathochromic shift in its spectrum relative to 2. The amine also

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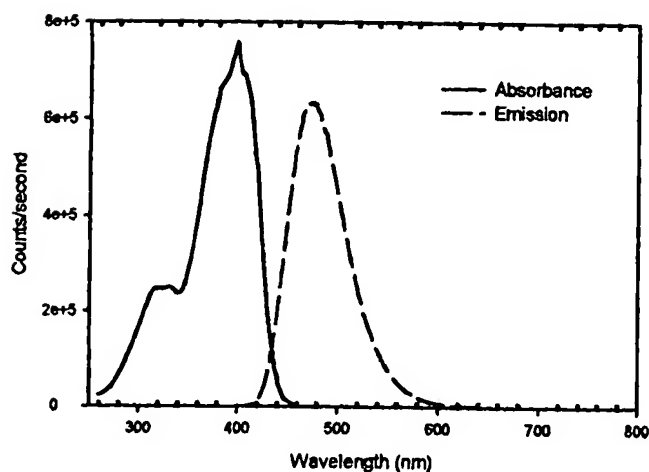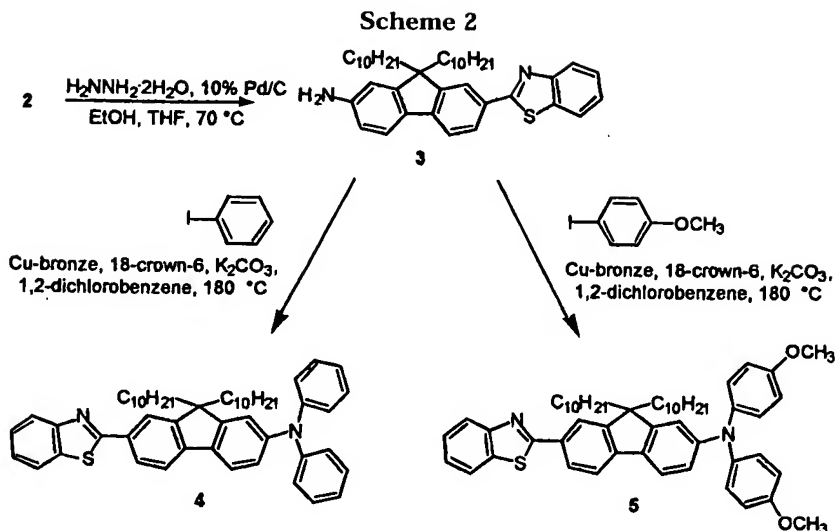
Scheme 2

Figure 1. Absorption and emission spectra of (7-benzothiazol-2-yl-9,9-didecylfluoren-2-yl)diphenylamine (4) in THF.

Figure 2. Absorption and emission spectra of (7-benzothiazol-2-yl-9,9-didecyl-fluoren-2-yl)bis(4-methoxyphenyl)amine (5) in THF.

displayed a brilliant blue fluorescence in solution upon irradiation with long wavelength UV radiation. The FT-IR spectrum of **3** revealed characteristic stretching absorptions for NH_2 (3741 and 3383 cm^{-1}) and benzothiazole C=N (1603 cm^{-1}). MS, ^1H, and ^{13}C NMR spectroscopic data provided additional structural confirmation of **3**, with the NH_2 resonances observed as a broad singlet at 3.82 ppm in the ^1H NMR spectrum and a concomitant upfield shift of the protons on C1 and C3 of the fluorene ring upon reduction of NO_2 to NH_2. Amine **3** was a key intermediate from which funcitonalization of the structure was performed using Ullmann amination reaction conditions. A new TPA dye was attained via a Cu-meditated Ullmann condensation of **3** with iodobenzene in *o*-dichlorobenzene using K_2CO_3 as base and 18-crown-6 at 180 °C (Scheme 2).[23] (7-Benzothiazol-2-yl-9,9-didecyl-fluoren-2-yl)diphenylamine **4** was obtained in 80% yield as a yellow viscous oil after column chromatographic purification. The FT-IR spectrum revealed the complete disappearance of the NH_2 stretching vibrations observed in **3**, likewise the NH_2 resonances were no longer observed in the ^1H NMR spectrum of **4**. The absorption and emission spectra of **4** are illustrated in Figure 1. The UV–vis absorption of **4** ranges from 239 to 450 nm with a λ_{max} of 392 nm. Fluorenylbenzothiazole **4** emitted
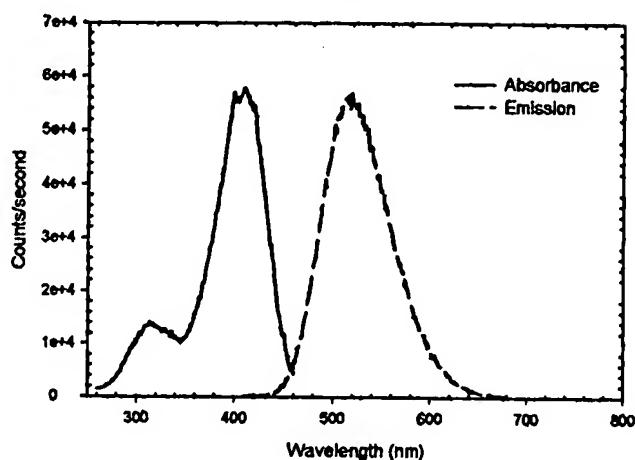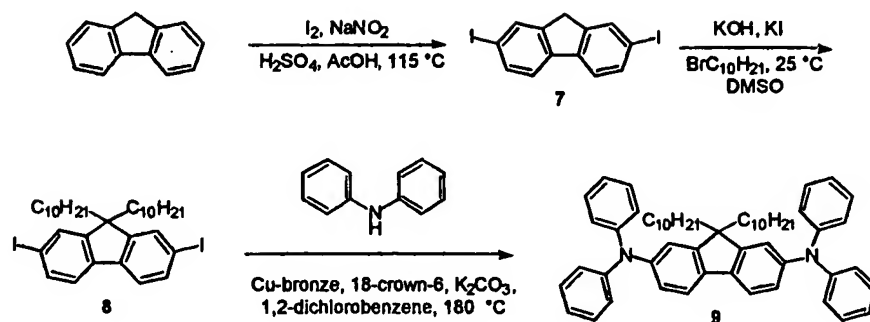
brilliant yellow-green fluorescence in solution upon exposure to long wavelength UV radiation. As can be seen, the fluorescence emission spectrum of **4** ranges from 400 to 630 nm with an emission λ_{max} of 475 nm upon excitation at 380 nm. Fluorene derivative **4** possesses highly desirable characteristics for use in two-photon fluorescence microscopy, i.e., since it has a λ_{max} of 392 nm.

Amine **3** was used to prepare another TPA dye with the aim to increase the electron density of the donor relative to that of **4**, producing a slight bathochromic shift in the absorption spectrum. Hence, **3** was subjected to similar Ullmann amination conditions with 4-iodoanisole, providing (7-benzothiazol-2-yl-9,9-didecylfluoren-2-yl)-bis-(4-methoxyphenyl)amine (**5**) as a bright yellow viscous oil in 82% yield after column chromatography (Scheme 2). CHN analysis was in good agreement with calculated values, and FT-IR spectroscopic analysis revealed the asymmetric and symmetric C—O—C stretches at 1241 and 1038 cm^{-1}, respectively. The ^1H NMR spectrum of **5** was similar to that of **4** with a major distinction of a singlet at 3.78 ppm, characteristic of the methoxy protons. A new signal was evident in the ^{13}C NMR spectrum at 55.3 ppm, ascribable to the methoxy carbons. Absorption and emission spectra for methoxy derivative **5** are presented in Figure 2. The UV—visible absorption of **5** ranged from 239 to 480 nm with a λ_{max} at 408 nm. The

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Scheme 3

fluorescence emission spectrum of **5** ranged from 410 to 675 nm with an emission λ_{max} of 516 nm upon excitation at 400 nm. Fluorene derivative **5** also possesses highly desirable characteristics for use in two-photon fluorescence microscopy, i.e., it has a λ_{max} of 408 nm.

7-Iodo-9,9-didecyl-2-nitrofluorene (**1**) was also employed as a key intermediate, whereby both the nitro and iodo groups in the 2 and 7 positions allowed for relatively easy derivitization of the fluorene ring system. The Ullmann amination conditions discussed above were applied to **1** with diphenylamine, affording (9,9-didecyl-7-nitro-fluoren-2-yl)diphenylamine **6** (Scheme 1) as an orange oil in ca. 80% yield. Fluorene **6** exhibited absorption from 220 to 520 nm with a λ_{max} of 300 nm in THF, and, as anticipated, no fluorescence detectable upon exposure to long wavelength UV light. CHN analysis was found to be in good agreement with calculated values, and the FT-IR spectrum displayed asymmetric and symmetric NO_2 stretches at 1519 and 1334 cm^{-1}, respectively.

The versatility of the Ullmann condensation reaction for the preparation of another dye of varying electronic character was demonstrated in the formation of 9,9-didecyl-2,7-bis-(N,N-diphenylamino)fluorene (**9**). The symmetrically situated bis-diphenylamino donor groups were incorporated at the fluorenyl 2 and 7 positions, affording a pale yellow crystalline solid in ca. 20% yield from fluorene. CHN analysis was found to be in excellent agreement with calculated values. Additional structural confirmation was secured by ^1H and ^{13}C NMR spectroscopic analysis, with broad resonances observed for aromatic protons from 6.7 to 7.30 ppm in the ^1H NMR spectrum. Also evident in the ^1H NMR spectrum were characteristic resonances of the decyl groups, with the six terminal methyl protons appearing as a triplet centered at 0.85 ppm, broad multiplets at 0.65, 1.05, and 1.20, corresponding to 32 of the 36 methylene protons, and the four methylene protons α to the fluorenyl benzylic carbon (C9) at 1.85 ppm. Evident in the absorption and emission spectra (Figure 3) was absorption from 240 to 410 nm with a λ_{max} of 378 nm, suitable for two-photon excitation at ca. 760 nm. Fluorene **9** exhibited fluorescence emission from 375 to 525 nm with an emission λ_{max} of 400 nm upon excitation at 370 nm. Two-photon absorption measurements are currently underway using a nondegenerate femtosecond pump/probe technique.[24] Preliminary data indicate that the fluorene derivatives prepared in this study have high two-photon absorptivity. For example, diphenylaminobenzothiazolyl-
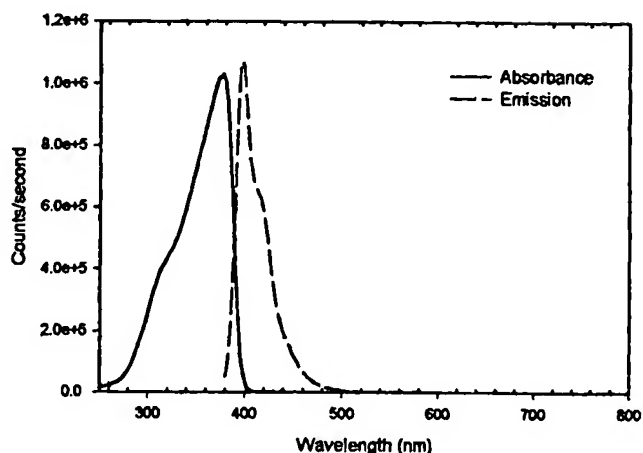

Figure 3. Absorption and emission spectra of 9,9-didecyl-2,7-bis(N,N-diphenylamino)fluorene (**9**) in THF.

fluorene **4** exhibited nondegenerate TPA in a pump probe experiment in which TPA was induced by spatial and temporal overlap of a 1210 nm femtosecond pump beam and a femtosecond white light continuum (wlc) probe beam. The wavelength of the pump beam was selected as 1210 nm (the photon energy at this wavelength is not energetic enough to cause degenerate TPA). At a probe wavelength of 615 nm from the wlc, the TPA cross section (δ) was ca. 820 × 10^{-50} cm^4 s photon^{-1} molecule^{-1}. This is consistent with δ values expected for molecules that undergo large dipole moment changes upon photoexcitation and compares quite favorably with δ's for recently reported chromophores with high two-photon absorptivity.[19,25] Details of nonlinear absorption spectroscopic analysis and quantum mechanical calculations of the derivatives reported herein will be reported elsewhere.

Conclusions

A strategy was developed in which the Ullmann amination reaction provided access to a number of fluorene analogues from common intermediates. The synthetic methodology facilitated facile functionalization at positions 2, 7, and 9 of the fluorene ring. Thus, efficient synthesis of key intermediates (**1** and **3**) afforded new TPA chromophores from fluorene. Systematic variation of molecular structure was accomplished, providing derivatives of varying electronic character. Through conversion of amine or iodofluorene derivatives, analogues bearing substitutents with varying electron-donat-

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ing and electron-withdrawing ability, e.g., diphenylamino, bis-(4-methoxyphenyl)amine, nitro, and benzothiazole, were prepared. The versatility of the Ullmann condensation reaction provided target compounds **4, 5, 6**, and **9** in reasonable yields. All target compounds were fully characterized, including mp, UV–vis, IR, elemental analysis, and ^{1}H and ^{13}C NMR, resulting in a series of well characterized, highly functionalized fluorene derivatives.

A remarkable feature of these materials is their solubility in common organic solvents (e.g., hexane, THF, CH_2Cl_2), due, in large part, to the decyl groups at the 9 position of the fluorene ring system. Unlike the unalkylated parent, most of the decylated derivatives were readily soluble in hexane. Target compounds **4, 5**, and **9** are potentially useful as fluorophores in two-photon fluorescence microscopy. Their UV–vis spectra display desirable absorption in the range of interest suitable for two-photon excitation by near-IR femtosecond lasers used in two-photon fluorescence imaging. Preliminary measurements of nonlinear absorption indicate select derivatives exhibit high two-photon absorptivity, affirming their potential as two-photon fluorophores. Current efforts are directed at correlation of the fluorene derivatives' molecular structure with nonlinear absorption, determination of their photophysical properties (e.g., fluorescence quantum yields), and their use in two-photon fluorescence imaging.

Experimental Section

General Methods. Reactions were conducted under N_2 or Ar atmospheres. THF was distilled over sodium before use. All other reagents and solvents were used as received from commercial suppliers. 2-Nitrofluorene,[17] 7-iodo-2-nitrofluorene,[18] and 2-(tri-*n*-butylstannyl)benzothiazole[19] were synthesized according to the respective literature preparations.

Synthesis of 7-Iodo-9,9-didecyl-2-nitrofluorene (1). A mixture of 7-iodo-2-nitrofluorene (9.6 g, 28 mmol), 1-bromodecane (12.58 g, 57 mmol), KI (0.49 g, 3 mmol), and DMSO (65 mL) was stirred at room temperature, to which powdered KOH (6.65 g, 119 mmol) was slowly added under N_2. The color of the reaction mixture changed from bright yellow to dark green immediately after KOH addition, and, after 1h, the reaction mixture was poured into water and extracted with hexanes. The organic extract was washed with water, dried over $MgSO_4$, and concentrated, affording 16.5 g of a viscous dark orange oil that crystallized upon standing. Purification was accomplished by column chromatography using silica gel (40:1 hexanes/ EtOAc), providing 13.6 g of yellow solid (77% yd, mp = 58.5–60 °C). MS (EI, 70 eV) *m/z*: 617 (M$^+$); 477 (M – $C_{10}H_{21}$); 476 (M – C_{10} H_{21}); 350 (M – $C_{19}H_{40}$); 336 (M – $C_{40}H_{41}$); 85 ($C_6H_{13}^+$); 71 ($C_4H_{11}^+$); 57 ($C_4H_9^+$); 43 ($C_3H_7^+$). Anal. Calcd for $C_{33}H_{48}INO_2$: C, 64.17; H, 7.83; I, 20.55; N, 2.27. Found: C, 64.47; H, 8.16; I, 20.40; N, 2.15. ^{1}H NMR (300 MHz, CDCl$_3$) δ: 8.25 (d, 1H), 8.18 (d, 1H), 7.79 (s, 1H), 7.72 (m, 2H), 7.53 (d, 1H), 1.99 (m, 4H), 1.18 (bm, 14H), 1.05 (bm, 14H), 0.83 (t, 6H), 0.56 (m, 4H). ^{13}C NMR (50 MHz, CDCl$_3$) δ: 154.4, 151.3, 147.5, 146.5, 138.3, 136.5, 132.5, 123.3, 122.6, 120.0, 118.2, 95.5, 55.9, 39.9, 31.8, 29.7, 29.4, 29.2, 29.1, 23.6, 22.6, 14.1.

Synthesis of 2-(9,9-Didecyl-7-nitrofluoren-2-yl)benzothiazole (2). Fluorene derivative 1 (10.1 g, 16 mmol) and 2-(tri-*n*-butylstannyl)benzothiazole (8.5 g, 20 mmol) were dissolved in 200 mL of toluene and degassed under vacuum and Ar. Pd(PPh$_3$)$_4$ (0.47 g, 0.4 mmol) was added and the mixture was degassed. The reaction mixture was heated to 110 °C under Ar. and found to be complete after about 4.5 h during which the mixture turned black in coloration. Toluene was removed in vacuo, and the resulting dark orange oil was passed through a Si-gel column (30:1 hexanes/EtOAc) and

recrystallized from hexanes affording 6.24 g of bright yellow crystals (61% yd, mp = 94–95 °C). MS (EI, 70 eV) *m/z*: 624 (M$^+$); 497 (M – C_9H_{19}); 483 (M – $C_{10}H_{21}$); 357 (M – $C_{19}H_{39}$); 343 (M – $C_{20}H_{41}$); 311 (M – $C_{20}H_{43}$ – NO); 297 (M – $C_{20}H_{42}$ – NO_2); 71 ($C_5H_{11}^+$); 57 ($C_4H_9^+$); 43 ($C_3H_7^+$). UV–vis (THF): λ_{max} = 365 nm (220–420 nm). Anal. Calcd for $C_{40}H_{48}N_2O_2S$: C, 76.88; H, 7.83; N, 4.48; S, 5.13. Found: C, 76.90; H, 8.41; N, 4.32; S, 5.14. ^{1}H NMR (200 MHz, CDCl$_3$) δ: 8.32, 8.37 (dd, 1H), 8.24, 8.18 (dd, 1H), 8.14 (m, 1H), 8.10 (m, 1H), 7.96, 7.92 (dm, 1H), 7.91, 7.88 (dd, 1H), 7.87, 7.84 (dd, 1H), 7.57, 7.38 (dd, 1H), 7.53, 7.42 (dm, 1H), 7.49, 7.46 (dd, 1H), 2.14 (m, 4H), 1.14 (bm, 14H), 1.04 (bm, 14H), 0.81 (t, 6H), 0.62 (m, 4H). ^{13}C NMR (50 MHz, CDCl$_3$) δ: 168.0, 154.4, 153.4, 152.9, 147.9, 146.7, 141.6, 135.3, 134.6, 127.8, 127.6, 126.7, 125.6, 123.6, 123.5, 122.0, 121.8, 120.6, 118.6, 56.4, 40.2, 32.0, 30.4, 30.0, 29.7, 29.4, 24.0, 22.8, 14.3. FT-IR (KBr, cm^{-1}): 3066 (ArCH), 2924, 2852 (alCH), 1589 (C=N), 1519 (NO$_2$ as), 1337 (NO$_2$ sy).

Synthesis of 7-Benzothiazol-2-yl-9,9-didecylfluoren-2-ylamine (3). Nitro compound 2 (4.0 g, 6.4 mmol) was dissolved in a mixture of 15 mL of EtOH and 15 mL of THF at room temperature. To this was added 0.2 g of 10% Pd/C, and the mixture was heated to 70 °C under Ar. Hydrazine hydrate (2 mL, 38 mmol) was added dropwise via syringe over 20 min. The reaction mixture was stirred for 12 h at 70 °C, cooled to room temperature, and concentrated, affording a yellow oil. Purification was accomplished by passing a hexane solution of the amine through a silica gel plug, eluting with hexanes, providing 3.6 g of viscous, sticky yellow oil (95% yd). MS (EI, 70 eV) *m/z*: 594 (M$^+$); 453 (M – $C_{10}H_{21}$); 327 (M – $C_{19}H_{39}$). 313 (m-$C_{20}H_{41}$); 163 ($C_{13}H_7^+$); 57 ($C_4H_9^+$); 43 ($C_3H_7^+$). UV–vis (THF): λ_{max} = 380 nm (210–450 nm). ^{1}H NMR (200 MHz, CDCl$_3$) δ: 8.09, 8.05 (dm, 1H), 8.04 (d, 1H), 7.99, 7.95 (dd, 1H), 7.90, 7.86 (dd, 1H), 7.63, 7.59, 7.56, 7.51 (dd, 1H), 7.51, 7.44, 7.39, 7.32, (dq, 1H), 7.48, 7.35 (dm, 1H), 7.19 (m, 1H), 6.67 (M, 2H), 3.82 (bs, 2H), 1.98 (m, 4H), 1.15 (bm, 14H), 1.04 (bm, 14H), 0.82 (t, 6H), 0.66 (m, 4H). ^{13}C NMR (50 MHz, CDCl$_3$) δ: 169.3, 154.5, 153.8, 150.8, 147.1, 145.1, 131.6, 130.8, 129.2, 128.4, 127.3, 126.4, 125.0, 123.0, 121.5, 121.5, 118.8, 114.3, 109.8, 55.4, 40.8, 32.1, 30.4, 30.3, 29.8, 29.7, 29.5, 24.0, 22.8, 14.3. FT-IR (KBr, cm^{-1}): 3741, 3383 (NH$_2$), 2926, 2855 (alCH), 1460 (ArC=C), 1603 (C=N).

General Procedure for the Ullmann Condensation Used for the Preparation of 4, 5, 6, and 9. Synthesis of (7-Benzothiazol-2-yl-9,9-didecylfluorene-2-yl)diphenylamine (4). The amine 3 (0.53 g, 0.891 mmol) was dissolved in 3 mL of 1,2-dichlorobenzene at room temperature under N_2. To this were added K$_2$CO$_3$ (0.99 g, 7.16 mmol), 18-crown-6 (0.07 g, 0.265 mmol), and copper bronze (0.28 g, 4.41 mmol) at room temperature, resulting in an orange solution. Iodobenzene (0.25 mL, 3.56 mmol) was added, and the reaction mixture was heated to 180 °C for 43 h. Upon completion, the dark brown mixture was filtered hot through a short silica gel plug and the yellow solution was concentrated resulting in an yellow-orange oil. The high boiling solvent 1,2-dichlorobenzene, and excess iodobenzene were removed under reduced pressure. The orange oil was further purified by column chromatography on silica gel using first 10:1:3 hexanes/EtOAc/THF, followed by 15:1 hexanes/EtOAc resulting in 0.53 g of a bright yellow-orange oil (80% yd). UV–vis (THF): λ_{max} = 392 nm (239–450 nm). Anal. Calcd for $C_{52}H_{62}N_2S$: C, 83.59; H, 8.36; N, 3.75. Found: C, 83.53; H, 8.47; N, 3.83. ^{1}H NMR (200 MHz, CDCl$_3$) δ: 8.13 (dm, 1H), 8.07 (dm, 1H), 7.04, 8.00 (dd, 1H), 7.93, 7.89 (dd, 1H), 7.51 (d, 1H), 7.63 (d, 1H), 7.54, 7.50, 7.49, 7.46 (dd, 1H), 7.42, 7.38, 7.37, 7.34 (1H), 7.31, 7.27 (dd, 4H), 7.16 (d, 1H), 7.13 (t, 2H), 7.07 (dm, 1H), 7.04 (dm, 4H), 1.90 (m, 4H), 1.20 (bm, 14H), 1.05 (bm, 14H), 0.85 (t, 6H), 0.70 (m, 4H). ^{13}C NMR (50 MHz, CDCl$_3$) δ: 168.7, 154.2, 152.9, 151.4, 147.9, 147.7, 144.0, 135.0, 134.8, 131.4, 129.1, 127.1, 126.1, 124.8, 124.0, 123.1, 122.8, 122.7, 121.4, 121.3, 120.9, 119.3, 118.7, 55.3, 40.1, 31.8, 29.9, 29.5, 29.2, 23.8, 22.6, 14.0. FT-IR (KBr, cm^{-1}): 3060 (ArCH), 2925, 2853 (alCH), 1595 (C=N), 1461 (ArC=C).

(7-Benzothiazol-2-yl-9,9-didecylfluoren-2-yl)-bis-(4-methoxyphenyl)amine (5). A bright yellow oil was obtained after column chromatographic purification (0.58 g, 82% yd).

UV−vis (THF): λ_{max} = 408 nm (239−480 nm). Anal. Calcd for $C_{54}H_{66}N_2O_2S$: C, 80.35; H, 8.224; N, 3.47. Found: C, 80.08; H, 8.20; N, 3.35.; ^{1}H NMR (200 MHz, CDCl₃) δ: 8.09 (dm, 1H), 8.04 (dm, 1H), 8.02, 7.97 (dd, 1H), 7.92, 7.88 (dd, 1H), 7.66 (d, 1H), 7.35 (d, 1H), 7.32, 7.49, 7.48, 7.45 (dd, 1H), 7.40, 7.37, 7.36, 7.33 (dd, 1H), 7.10, 7.06 (dd, 1H), 6.98 (d, 1H), 6.92, 6.85 (dd, 4H), 8.88, 6.82 (dd, 4H), 3.78 (s, 6H), 1.90 (m, 4H), 1.20 (bm, 14H), 1.05 (bm, 14H), 0.85 (t, 6H), 0.75 (m, 4H). ^{13}C NMR (50 MHz, CDCl₃) δ: 169.0, 155.7, 154.2, 152.8, 151.2, 150.0, 144.3, 141.2, 134.9, 133.1, 130.9, 127.1, 126.1, 124.8, 122.8, 121.4, 121.3, 120.7, 118.9, 115.8, 114.6, 55.3, 55.2, 40.1, 31.8, 29.8, 29.5, 29.3, 23.8, 22.6, 14.1. FT-IR (KBr, cm⁻¹): 3060 (ArCH), 2925, 2853 (alCH, methyl), 1603 (ArC=C), 1241 (C−O−C as), 1038 (C−O−C sy).

Synthesis of (9,9-Didecyl-7-nitrofluoren-2-yl)diphenyl-amine (6). An orange oil was obtained (4 g, 93% yd). UV−vis (THF): λ_{max} = 300 nm (220−520 nm). Anal. Calcd for $C_{45}H_{58}N_2O_2$: C, 82.02; H, 8.87; N, 4.25.; Found: C, 82.15; H, 8.95; N, 4.05. ^{1}H NMR (200 MHz, CDCl₃) δ: 8.25, 8.22 (dd, 1H), 8.14 (d, 1H), 7.67 (d, 1H), 7.62 (d, 1H), 7.32, 7.29 (dd, 4H), 7.16 (d, 1H), 7.10 (m, 2H), 7.06 (dm, 1H), 7.03 (dm, 4H), 1.85 (m, 4H), 1.20 (bm, 14H), 1.05 (bm, 14H), 0.85 (t, 6H), 0.65 (m, 4H). ^{13}C NMR (50 MHz, CDCl₃) δ: 153.9, 151.6, 149.3, 147.6, 147.5, 146.2, 132.8, 129.3, 124.6, 123.5, 123.4, 122.6, 121.9, 118.7, 118.0, 117.8, 55.8, 39.9, 31.9, 29.8, 29.5, 29.3, 23.8, 22.6, 14.1. FT-IR (KBr, cm⁻¹): 3060, 3038 (ArCH), 2923, 2852 (alCH), 1519 (NO₂ as), 1334 (NO₂ sy).

Synthesis of 9,9-Didecyl-2,7-bis(*N*,*N*-diphenylamino)-fluorene (or 2,7-Dibenzhydryl-9,9-didecyl-fluorene) (9). Fluorene was diiodinated using similar iodination conditions employed previously to yield crystalline 2,7-diiodofluorene (**7**, mp = 206−210 °C). ^{1}H NMR (200 MHz, CDCl₃) δ: 7.89 (s, 2H), 7.70 (d, 2H), 7.50 (d, 2H), 3.85 (s, 2H). Compound **7** was didecylated using the previous alkylation conditions to generate 9,9-didecyl-2,7-diiodofluorene (**8**) as a yellow oil. ^{1}H NMR (200 MHz, CDCl₃) δ: 7.65 (d, s, 3H), 7.40 (d, 2H), 1.85 (m, 4H), 1.20 (bm, 14H), 1.05 (bm, 14H), 0.85 (t, 6H), 0.55 (m, 4H). The alkylated diiodofluorene **8** was subjected to similar Ullmann condensation reaction with diphenylamine to yield the desired product **9** as off-white crystals (20% yd from fluorene, mp = 81.5−83.5 °C). Anal. Calcd for $C_{57}H_{68}N_2$: C, 87.64; H, 8.77; N, 3.58. Found: C, 87.27; H, 8.77; N, 3.58. ^{1}H NMR (200 MHz, CDCl₃) δ: 6.70−7.30 (bm, 26H), 1.85 (m, 4H), 1.20 (bm, 14H), 1.05 (bm, 14H), 0.85 (t, 6H), 0.65 (m, 4H). ^{13}C NMR (50 MHz, CDCl₃) δ: 151, 145, 128, 123, 121.9, 119, 39, 39.9, 31.9, 29.8, 29.5, 29.3, 23.8, 22.6, 14.1.

Supporting Information Available: ^{1}H and ^{13}C NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Multiphoton-absorbing organic materials for microfabrication, emerging optical applications and non-destructive three-dimensional imaging

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ABSTRACT: Non-resonant two-photon absorption (TPA) can be defined as the simultaneous absorption of two photons, via a virtual state, in a medium. TPA exhibits a quadratic dependence of absorption on the incident light intensity, resulting in highly localized photoexcitation. Recent developments in the design and synthesis of efficient, stable TPA organic materials are discussed. Microfabrication via two-photon induced free radical polymerization of acrylate monomers and cationic polymerization of epoxide monomers was accomplished using commercially available photoinitiators, and also a custom-made compound possessing high two-photon absorptivity. Two-photon facilitated photoisomerization of a fulgide in solution and in a polymer thin film demonstrated two-photon induced photochromism and its application in interferometric image recording, respectively. Greatly enhanced signal-to-noise ratios and resolution were achieved in the non-destructive three-dimensional two-photon fluorescence imaging of a polymer-coated substrate versus conventional single-photon laser scanning confocal microscopic imaging. Multifunctional TPA organic materials and fabrication of functional microstructures are also discussed. Copyright © 2000 John Wiley & Sons, Ltd.

KEYWORDS: two-photon absorption; microfabrication; photoisomerization; photo-polymerization; fluorescence imaging

INTRODUCTION

Organic materials exhibiting significant non-linear responses to applied electric or electromagnetic fields have attracted intense interest during the last few decades. Currently, organic materials occupy a prominent role in 2-D (linear) display technology based on specifically induced phase transitions in liquid crystalline materials, thereby altering their optical properties, and in the rapidly developing arena of organic light-emitting diodes (electroluminescent materials). Organic chromophores are becoming an integral component in second and third harmonic generation, devices for radiation frequency conversion and waveguides. Suitable materials for such applications manifest a non-linear optical response in the presence of an applied electric field, resulting in amplification of the particular optical property (e.g. refractive index change) relative to that obtained via a

linear dependence. The end of the 20th century and the beginning of the 21st century have been accompanied by an ever pressing need for materials that exhibit amplification or respond in a highly non-linear manner to a particular stimulus. In particular, compounds that undergo strong non-linear, multiphoton absorption are being investigated as materials for a wide variety of potential applications in areas ranging from optical information storage, 3-D optical memories, biophotonics, materials science and photochemistry. For example, it is projected that a multiphoton-based 3-D optical volumetric memory will provide up to three orders of magnitude more information in the same size enclosure relative to a 2-D optical disk memory.[1]

The non-linear, multiphoton process of two-photon absorption (TPA) has been gaining greater interest among a number of multidisciplinary areas, particularly in the rapidly developing fields of multiphoton fluorescence imaging, optical data storage and switching, optical sensor protection, telecommunications, laser dyes, 3-D microfabrication and photodynamic therapy (PDT).[2–5] The demands of such applications exceed properties and reliabilities delivered by current organic materials, underscoring the need for increasingly sophisticated non-linear optical organic materials. Since the probability of a TPA process is proportional to the square of the

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incident light intensity, photoexcitation is spatially confined to the focal volume.[1] Such precise control of photoexcitation is intriguing, facilitating the development of new technologies, processes and materials that require 3-D spatial resolution of physical properties, both static (permanent) and dynamic (reversible).

The theory of the simultaneous absorption of two photons was developed by Goeppert-Mayer in 1931,[6] but remained mainly an intellectual curiosity until the advent of the pulsed laser providing very high-intensity light. For simplicity, two-photon absorption can be conceptualized from a semiclassical perspective.[1] In the TPA process, molecules exposed to high intensity light can undergo near simultaneous absorption of two photons mediated by a so-called 'virtual state,' a state with no classical analog. The combined energy of the two photons accesses a stable excited state of the molecule. If the two photons are of the same energy (wavelength), the process is referred to as degenerate TPA. On the other hand, if the two photons are of different energy (wavelength), the process is non-degenerate TPA.

As light passes through a molecule, the virtual state may form, persisting for a very short duration (of the order of a few femtoseconds). TPA can result if a second photon arrives before decay of this virtual state, with the probability of TPA scaling with the square of the light intensity. This process is generally termed simultaneous two-photon absorption. Two-photon absorption thus involves the *concerted* interaction of both photons that combine their energies to produce an electronic excitation analogous to that conventionally caused by a single photon of a correspondingly shorter wavelength. Unlike single-photon absorption, whose probability is linearly proportional to the incident intensity, the TPA process depends on both a spatial and temporal overlap of the incident photons and takes on a quadratic (non-linear) dependence on the incident intensity.

Two-photon transitions can be described by two different mechanistic types. For non-polar molecules with a low-lying, strongly absorbing state near the virtual level, only excited states that are forbidden by single-photon selection dipole rules can be populated via two-photon absorption (Type 1 in Fig. 1).[1] The probability that this low-lying state can contribute to the virtual state is predicted by Heisenberg's uncertainty principle, with a virtual state lifetime approximated as $h/(4\pi\Delta E)$, where h is Planck's constant and ΔE is the energy difference between the virtual and actual states. Using this equation, it is predicted that an allowed state can contribute to formation of the virtual state for time $t_{virtual}$ which is equal to about $h/(4\pi\Delta E)$ with the transition probability proportional to $\Delta\mu^2$. For example, a lifetime of ca 0.3 fs is estimated for a state separation of 0.1 eV. Two-photon absorptivity, δ, is expressed in Goeppert-Mayer units (GM), with 1 GM = 1 × 10⁻⁵⁰ cm⁴ s molecule⁻¹ photon⁻¹. Molecules which undergo strong TPA via the Type 1 process have two-photon absorptivities up to 10 GM.[7]
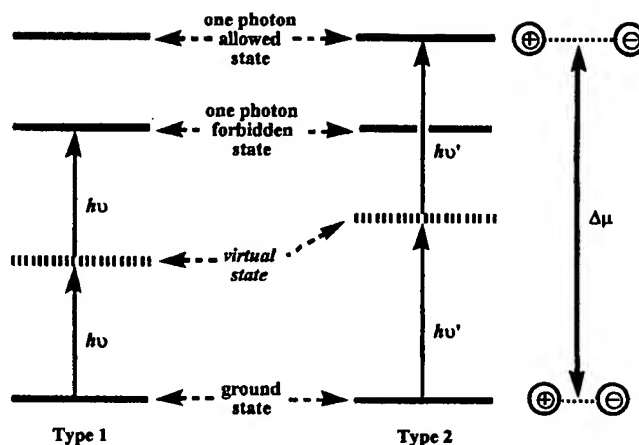

Figure 1. 'Simultaneous' two-photon absorption processes (Type 1 and 2)

In contrast, strong TPA can occur in polar molecules by a different mechanism (Type 2 in Fig. 1) in which a large change in dipole moment ($\Delta\mu > 10$ D) occurs upon excitation of the ground to an excited state.[1] Single-photon allowed states can then be accessed via TPA, and the virtual state lifetime is proportional to $\Delta\mu^2$, while the transition probability scales with $\Delta\mu^4$. In this case, both the ground and excited states can participate in the formation of the virtual state, enhancing TPA. In polar molecules with large $\Delta\mu$ between the ground and excited states, δ values in excess of 100 GM have been reported.[1,8]

The two-photon advantage

The quadratic, or non-linear, dependence of two-photon absorption on the intensity of the incident light has substantial implications. For example, in a medium containing one-photon absorbing chromophores, significant absorption occurs all along the path of a focused beam of suitable wavelength light. This can lead to out-of-focus excitation. In a two-photon process, negligible absorption occurs except in the immediate vicinity of the focal volume of a light beam of appropriate energy. This allows spatial resolution about the beam axis as well as radially, which circumvents out-of-focus absorption and is the principle reason for two-photon fluorescence imaging.[9] Particular molecules can undergo upconverted fluorescence through non-resonant two-photon absorption using near-IR radiation, resulting in an energy emission greater than that of the individual photons involved (up-conversion). The use of a longer wavelength excitation source for fluorescence emission affords advantages not feasible using conventional UV or visible fluorescence techniques, e.g. deeper penetration of the excitation beam and reduction of photobleaching.

Argon ion (488 nm) and frequency-doubled Nd:YAG (532 nm) lasers are the commonly used light sources for
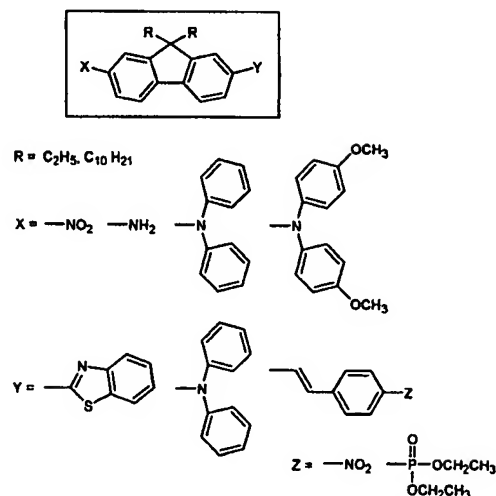
conventional (single-photon) laser scanning confocal microscopy (owing to their ready availability and low cost). Such light sources require fluorophores with strong absorbance near these wavelengths. Two-photon laser scanning fluorescence microscopy systems, on the other hand, are generally configured with a Ti:sapphire laser with 80–120 fs pulse output in the near-IR region (700–900 nm). Consequently, many typical single-photon fluorophores undergo only weak TPA in this region, since a fluorophore with λ_{max} of 490 nm would be expected to undergo two-photon absorption at ca 980 nm (a wavelength where the output power of commercial Ti:sapphire lasers is practically too low to be useful). Thus, commercial fluorophores are far from being optimized for use in two-photon fluorescence microscopy. A more reasonable absorption maximum for such chromophores is 380–420 nm (facilitating the use of near-IR femtosecond sources in the range 760–840 nm), since the TPA λ_{max} will be approximately twice the wavelength of the single-photon λ_{max}.[8]

RESULTS AND DISCUSSION

Two-photon absorbing compounds

Recent developments in the design of organic materials with large multiphoton absorptivity are based largely on the Type 2 TPA process outlined in Fig. 1, involving molecules that undergo large changes in dipole moment upon excitation from the ground to an excited state. A relatively limited number of two-photon absorbing compounds based on this premise have been reported. Among efficient TPA polar organic compounds are those with polarizable π-conjugated systems such as those reported with phenylethenyl,[10] fluorenyl[8,11–13] or polyenyl (bacteriorhodopsin and its analogs)[1] constructs bearing electron-donating (D) and/or electron-withdrawing (A) moieties, separated by a conjugated π-electron system, i.e., A–π–A, D–π–A, D–π–D. Femtosecond two-photon absorptivities for some of these materials are in the range 10–1000 GM.

As part of a program to establish an empirical body of non-linear absorptivity as a function of molecular structure, and to prepare efficient two-photon absorbing fluorophores, we have been investigating a series of compounds with systematic variation in molecular structure (Fig. 2).[8,11,12] Polar organic compounds such as 4-nitroaniline and 4-amino-4'-nitrobiphenyl undergo increases in dipole moments on photoexcitation from 6 to 14 D and from 6 to 20 D, respectively.[14] Thus, such compounds are expected to possess high two-photon absorptivity, as discussed above. We chose the fluorenyl ring system to serve as a thermally and photochemically stable π-conjugated analog of the 4,4'-disubstituted biphenyl derivatives. Locking the biphenyl unit into the fluorenyl ring provides greater electron delocalization

Figure 2. Structures of selected fluorene derivatives that undergo TPA and up-converted fluorescence

Fluorene Derivative	R	X	Y
1	C_2H_5	NPh_2	Z = 4-phosphonostyryl
2	C_2H_5	NPh_2	Z = 4-nitrostyryl
3	$C_{10}H_{21}$	NO_2	2-benzothiazolyl
4	$C_{10}H_{21}$	NH_2	2-benzothiazolyl
5	$C_{10}H_{21}$	NPh_2	2-benzothiazolyl
6	$C_{10}H_{21}$	$N(C_6H_4$-p-$OCH_3)_2$	2-benzothiazolyl
7	$C_{10}H_{21}$	NO_2	NPh_2
8	$C_{10}H_{21}$	NPh_2	NPh_2

through increased π molecular orbital overlap between the rings, enhancing molecular polarizability. Importantly, fluorene can be readily functionalized in the 2-, 7- and/or 9-positions. In addition to TPA optimization, another important molecular design aspect is wavelength sensitivity. Since commercially available Ti:sapphire lasers (output from 700 to 980 nm) are currently the most suitable light sources to provide the requisite high intensities for TPA, this must be considered in the design of TPA materials.

In order to prepare a series of derivatives for non-linear absorption studies, we sought the efficient preparation of key intermediates using synthetic methodology that would be readily adaptable for rapid functionalization. Ullmann condensation reactions of arylamines with aryl iodides and efficient Pd-catalyzed Heck and Stille coupling were employed to prepare a series of fluorene derivatives of varying electronic characteristics (Fig. 2).[8,11,12,15] The UV–visible absorption spectrum of phosphorylated fluorene derivative 1 in CH_3CN extended out to about 480 nm with two λ_{max}, one at 308 nm and the other at 383 nm. The visible absorption of nitro-containing fluorene derivative 2 in CH_3CN extended out to about 550 nm with two λ_{max}, one at 309 nm and the other at 414 nm. The fluorescence emission spectrum of 5 ranges from 400 to 630 nm with an emission λ_{max} of 475 nm upon excitation at 380 nm. Fluorene derivative 5 possesses highly desirable characteristics for use in
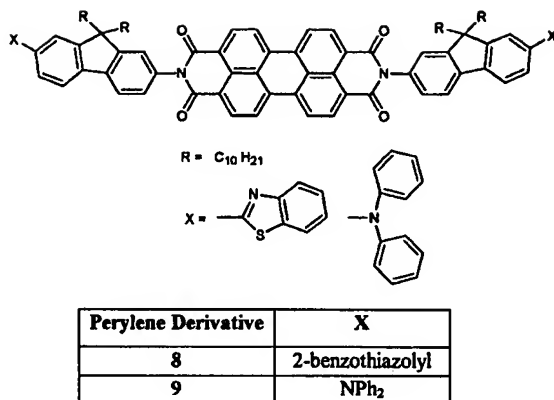
Perylene Derivative	X
8	2-benzothiazolyl
9	NPh₂

Figure 3. Structures of multifunctional TPA dyes with an electron-accepting perylene core
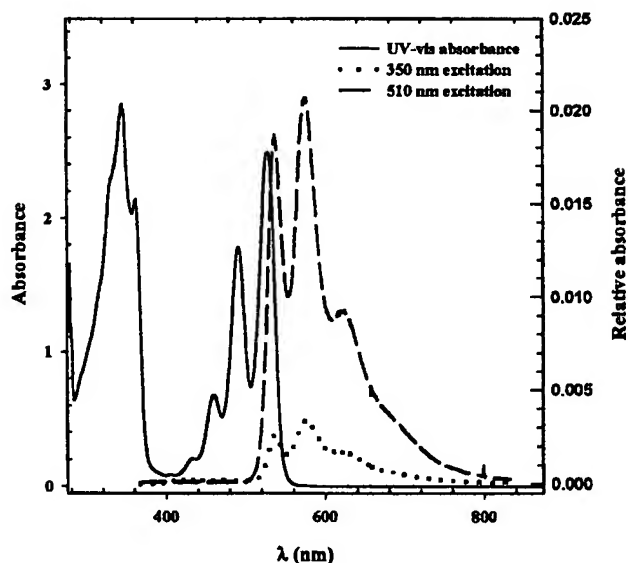

λ (nm)

Figure 4. Overlay of UV–visible absorption spectrum (solid line) and fluorescence emission spectra of perylenediimide **8** in THF excited at 350 nm (dotted line) and 510 nm (dashed line)

two-photon fluorescence microscopy, since it has an absorption λ_{max} of 392 nm. The fluorescence emission spectrum of **6** ranged from 410 to 675 nm with an emission λ_{max} of 516 nm upon excitation at 400 nm. Fluorene derivative **6** also possesses highly desirable characteristics for use in two-photon fluorescence microscopy, i.e. it has an absorption λ_{max} of 408 nm.

Two-photon absorption measurements have been performed on several of these compounds using an NLO spectrometer and pump–probe experiment. Details of the experimental technique have been described elsewhere.[11] Briefly, non-degenerate TPA was measured using a pump–probe experiment in which TPA was induced by spatial and temporal overlap of a 1210 nm femtosecond pump beam and a femtosecond white light continuum (WLC) probe beam. The wavelength of the pump beam was selected as 1210 nm (the photon energy at this wavelength is not energetic enough to cause degenerate TPA). The probe beam consisted of a femtosecond white light continuum (WLC) generated by irradiation of a sapphire window in front of the sample.

Preliminary data indicate that the fluorene derivatives in Fig. 2 have high two-photon absorptivity. For example, at a WLC probe wavelength of 615 nm, diphenylamino-benzothiazolylfluorene (**5**) exhibited nondegenerate TPA of ca 820 × 10⁻⁵⁰ cm⁴ s photon⁻¹ molecule⁻¹ (820 GM). Fluorene **1** exhibited a maximum two-photon absorptivity of ca 650 × 10⁻⁵⁰ cm⁴ s photon⁻¹ molecule⁻¹ (650 GM) at WLC wavelength 605 nm, whereas the two-photon absorptivity of fluorene **2** was significantly higher, ca 1300 × 10⁻⁵⁰ cm⁴ s photon⁻¹ molecule⁻¹ (1300 GM) at WLC wavelength 670 nm. Evident from this comparison is an increase in TPA with a more polar molecular structure, indicative of a larger $\Delta\mu$ for **2** relative to **1**, and consistent with δ values expected for molecules that undergo large dipole moment changes upon photoexcitation. The large two-photon absorptivities and associated λ_{max} for the fluorene derivatives open the door for potential applications of these chromophores

in multiphoton fluorescence imaging and other optical applications (as discussed below).

Next-generation multifunctional TPA materials are envisioned which incorporate functionality for high two-photon absorptivity along with molecular constructs for energy transfer, electron transport and/or morphological variation (Fig. 3). Driving our studies are potential applications of perylenediimide derivatives in molecular electronics applications such as organic photorefractive media for optical signal processing, electron-transporting components in organic light-emitting diodes, materials for electrophotography, n-type photovoltaic materials for solar energy conversion, fluorescent dyes and near-IR dyes.[15] We recently reported the synthesis of perylene-diimide **8**.[15] Interestingly, perylenediimide **8** exhibited two strong UV–visible absorption bands (Fig. 4), one in the UV from 270 to 385 nm (λ_{max} = 345 nm) and another in the visible from 410 to 545 nm with λ_{max} at 457, 486 and 522 nm. The first absorption band is due to the fluorenyl groups, as it is similar to the absorption range exhibited by the aminofluorene **4** from 210 to 450 nm with λ_{max} = 380 nm, while the absorption in the visible range is due to the central perylene ring system.

The fluorescence emission spectrum of perylene **8** was obtained and is shown in Fig. 4, where excitation was performed at λ_{ex} = 325 nm, resulting in an unexpected emission λ_{max} at 540, 585 and 625 nm. Excitation at λ_{ex} = 510 nm resulted in a similar emission λ_{max} profile to that obtained with the λ_{ex} = 325 nm.[15]

The resulting emission profile from the first excitation (350 nm) is indicative of an intramolecular energy transfer process whereby the excited fluorenyl group undergoes energy transfer to the perylene ring system,

facilitating excitation followed by emission.[15] This then generates a similar emission spectrum to that obtained from λ_{ex} = 510 nm, although the relative intensity of the fluorescence from this energy transfer process is weaker than that of the corresponding fluorescence obtained from the excitation performed at the visible maxima owing, presumably, to competing deactivation processes. Since the longer wavelength absorption band at the perylene moiety overlaps with its emission, reabsorption of the emission in the range 500–560 nm is likely. The bisdiphenylaminoperylene derivative 9 (Fig. 3) exhibited similar luminescence behavior to the benzothiazole analog but striking differences in morphology were observed. Whereas the benzothiazole analog 8 was an amorphous glass with T_g = 111 °C, the diphenylamino derivative 9 afforded large single crystals. DSC analysis of 9 revealed two melting endotherms at 160 and 297 °C, with corresponding cooling exotherms at 128 and 242 °C, respectively, demonstrating a degree of morphological control. We are currently investigating the basis of the morphology differences for these two derivatives through computational modeling.

Two-photon microfabrication

It is widely believed that a revolution in miniaturization, particularly in the field of microelectromechanical systems (MEMS), is under way. Figures predict the world's MEMS market to be more than $14 billion.[16] It is projected that the design and manufacturing technology that will be developed for MEMS may rival, or even surpass, the far-reaching impact of ICs on society and the world's economy. At the forefront of techniques being explored for 3-D spatially resolved materials imaging and processing are methods based on TPA. In contrast to the linear dependence of single-photon absorption on incident light intensity in conventional photopolymerization, the quadratic dependence of photoexcitation on light intensity in TPA can be exploited to confine polymerization to the focal volume and achieve fabrication of microstructures via 3-D spatially resolved polymerization.[5]

Two-photon absorption can occur in certain materials at wavelengths well beyond that which monomers, polymers and most organic substances absorb (one-photon), affording a greater depth of penetration, creating little or no damage to the host. Hence two-photon microfabrication via photoinitiated polymerization represents a potentially versatile technology that should be compatible with construction of mechanical, chemical, electrical, optical or biosensor systems. Additionally, it should be integratable with conventional IC processing technologies and femtosecond laser micromachining.

Although several reports of two-or multiphoton induced polymerization appeared in the literature as early as 1971,[17] most of these involve two or more

sequential, resonant single-photon excitation processes at single or multiple wavelengths (i.e. excited state absorption).[18–20] The resonant processes must be distinguished from the simultaneous two-photon excitation process of concern here owing to the fundamental differences in achieving spatially resolved polymerization, i.e. a much higher degree of inherent 3-D spatial resolution in the simultaneous process. Less relevant to the current objectives are a limited number of accounts of two-photon induced polymerization in the gas phase.[21–24]

Two-photon photopolymerization of commercial acrylate monomer systems, preformulated with UV photoinitiators, has been reported, although little information regarding photoinitiators was available.[25–28] Two-photon absorbing compounds based on phenylethenyl constructs bearing electron-donating and/or electron-withdrawing moieties have been reported.[10] Among these are electron-rich derivatives that have been found to undergo a presumed two-photon induced electron transfer to acrylate monomers[29] or proposed fluorescence energy transfer to a photoinitiator,[30] initiating polymerization. The reportedly efficient two-photon photoinitiators, although more photosensitive than previously studied UV photoinitiators, are not commercially available and require involved syntheses. Hence the practicality of their broader use is questionable.

Recently, we reported the near-IR two-photon induced polymerization of (meth)acrylate monomers using a commercially available photoinitiator system based on a visible light-absorbing dye.[5] Two-photon initiated polymerization was conducted at 775 nm via direct excitation of a commercially available dye (5,7-diiodo-3-butoxy-6-fluorone, H-Nu 470) in the presence of an arylamine, and (meth)acrylate monomer. The fluorone dye, 5,7-diiodo-3-butoxy-6-fluorone (H-NU 470), and N, N-dimethyl-2,6-diisopropylaniline were obtained from Spectra Group; thick films were cast on glass slides from neat mixtures and thin films were obtained via spin coating from a dioxane solution, resulting in an electron-transfer free radical initiation process. The excitation wavelength was well beyond the linear absorption spectrum for 5,7-diiodo-3-butoxy-6-fluorone (strong and weak absorption maxima at 330 and 470 nm, respectively). Four commercial acrylate and methacrylate monomer systems were used: ethoxylated bisphenol A diacrylate (SR349, Sartomer), pentaacrylate ester (SR9041, Sartomer), aromatic urethane acrylate blended with tripropylene glycol diacrylate (CN973A80, Sartomer), and 2-methyl-2-propenoic acid (1-methyethylidene)-bis(4,1-phenyleneoxy-2-hydroxy-1,3-propanediyl) ester (BisGMAx950, Esschem).

According to the mechanism deduced from single-photon photochemical studies of the initiating system (Fig. 5),[31] electron transfer from the aromatic amine (N,N-dimethyl-2,6-diisopropylaniline, DIDMA) to the fluorone derivative, followed by proton transfer from the amine to the fluorone, resulted in formation of an
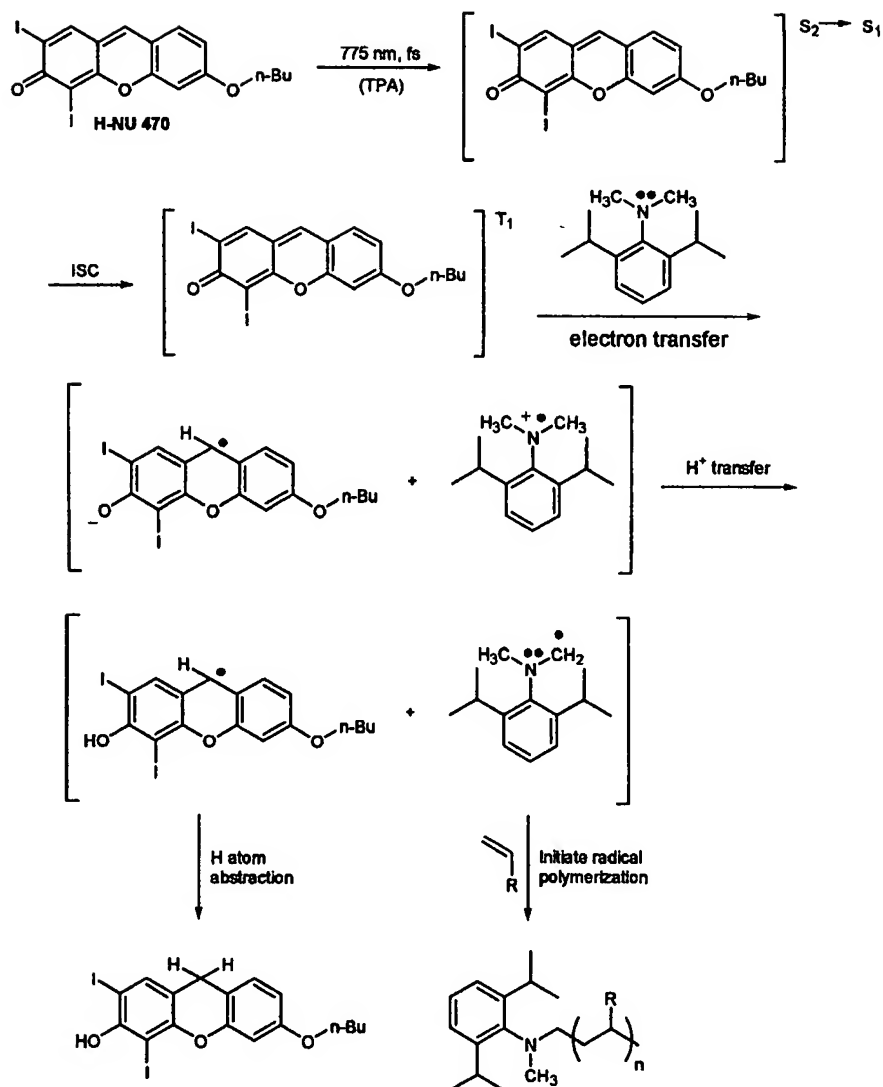
Figure 5. Direct two-photon photoinitiated polymerization at 775 nm

arylamine bearing a free radical localized on the α-methylene carbon. This free radical species then initiated polymerization of (meth)acrylate derivatives. A diaryliodonium salt can be added to accelerate the rate of polymerization.

A number of control experiments were performed to support a two-photon based excitation process. First, experiments were performed using a Ti:sapphire laser in continuous wave (CW) vs mode-locked (80 fs pulse width). Polymerization was not observed in CW mode, while polymerization occurred only when the laser was mode-locked. Next, experiments were performed on monomer alone (no initiator), in which no polymerization was observed upon exposure to near-IR femtosecond radiation. Furthermore, an initiator system comprised of isopropylthioxanthone (ITX) and DIDMA (monomer:-ITX:DIDMA mole ratio $1:1.4 \times 10^{-3}:5.4 \times 10^{-3}$) also afforded polymer in the presence of an acrylate monomer under near-IR femtosecond irradiation, attesting to the

generality of the electron transfer polymerization discussed above. Similarly, no polymer was produced when the ITX–DIDMA–acrylate mixture was exposed to the same wavelength in CW mode.

The formation of polymeric microstructures with a variety of dimensions was accomplished with the H-NU 470 initiator–acrylate monomer system [Fig. 7(a)]. Similar 2-D microstructures were obtained via two-
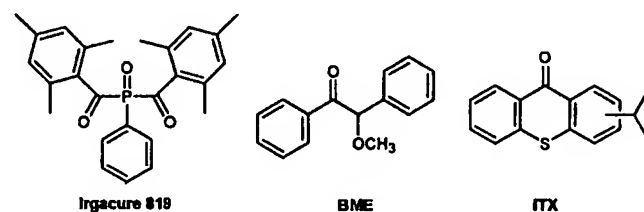

Figure 6. Free-radical photoinitiators for near-IR two-photon polymerization
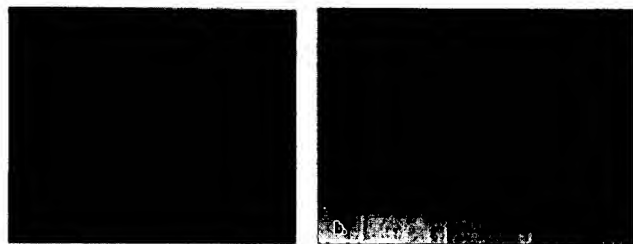
Figure 7. Optical micrographs of grid-type microstructures created via two-photon polymerization of an acrylate monomer (SR349) (a) using the H-NU 470 initiating system (9 μm linewidth, 100 μm line spacing) and (b) using fluorene 5 as initiator (18 μm linewidth, ~100 μm line spacing)

via TPE at 775 nm, presumably by means of an electron-transfer process. The resulting microstructure using fluorene 5 and SR349 acrylate had 18 μm linewidths of uniform spacing [Fig. 7(b)]. In a simple grid scan, linewidths of 9 μm, spaced 100 μm apart, were produced with the H-NU 470 system. Microstructures were readily examined by optical reflection microscopy.

A logical frontier is the preparation of functional microstructures. The fabrication of magnetically actuated microstructures via two-photon polymerization is envisioned. This was demonstrated via two-photon free radical polymerization of an acrylate monomer (SR349), with the H-NU 470–DIDMA initiator system, containing emulsion-stabilized magnetite nanoparticles. Optical microscope and AFM imaging revealed the microstructure and the presence of the magnetite nanoparticles entrapped in the polymeric microstructure (Fig. 8). In a polymerized grid-type microstructure containing magnetite nanoparticles, the optical micrograph revealed a structure with 18 μm horizontal linewidth. AFM topographic imaging of a small section of the microgrid was accomplished in broad band mode (50 kHz vibration), indicating a 300 nm average thickness of the polymer. AFM imaging in error mode (50 kHz vibration) showed a relatively even distribution of the nanometallic particles in the polymerized and unpolymerized areas.

Cationic photoinitiated polymerization of epoxides, vinyl ethers and methylenedioxolanes has received increasing attention, owing in large part to the oxygen insensitivity of the cationic process.[32] Commercially available diaryliodonium (CD-1012, Sartomer) and

photon polymerization of acrylate monomers using commercial photoinitiators and a 775 nm femtosecond laser. In particular, ary ketone photoinitiators (Fig. 6) such as isopropylthioxanthone (ITX), benzoin methyl ether (BME) and an acylphosphine oxide (Irgacure 819, CIBA) were found to be effective initiators (all have λ_{max} <400 nm), resulting in well-defined microstructures. Typical monomer:initiator molar ratios were $1{:}3.5 \times 10^{-4}$ (ITX), $1{:}7.5 \times 10^{-3}$ (BME), $1{:}1.1 \times 10^{-3}$ (Irgacure 819) and $1{:}1.9 \times 10^{-3}$ (fluorene 5, Fig. 2). In addition, compositions comprised of acrylate monomer SR349, ITX and N-ethyldiethanolamine, or SR349, H-NU 470 and N-ethyldiethanolamine in $1{:}3.2 \times 10^{-3}{:}0.36$ or $1{:}7.2 \times 10^{-4}{:}0.32$ molar ratios, respectively, were effective in forming microstructures upon two-photon excitation (TPE).

The strong two-photon absorbing compound fluorene 5 was an effective initiator for an acrylate polymerization
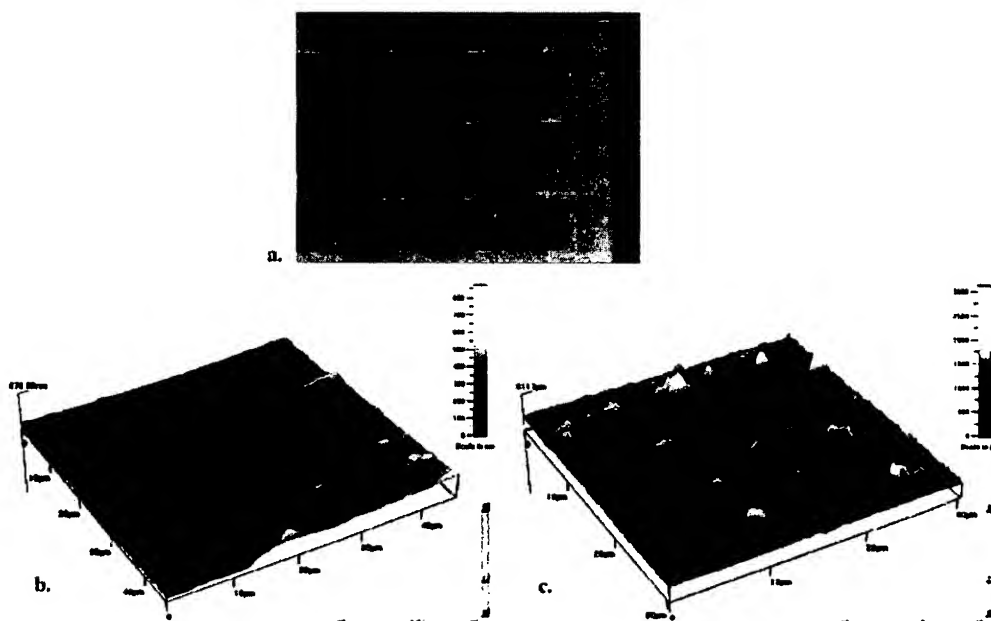

Figure 8. Polymerized grid-type microstructure containing magnetite nanoparticles: (a) optical micrograph (18 μm horizontal linewidth), (b) AFM topographic image of partial microgrid in broad band mode (50 kHz vibration) and (c) nanometallic particles in error mode (50 kHz vibration)
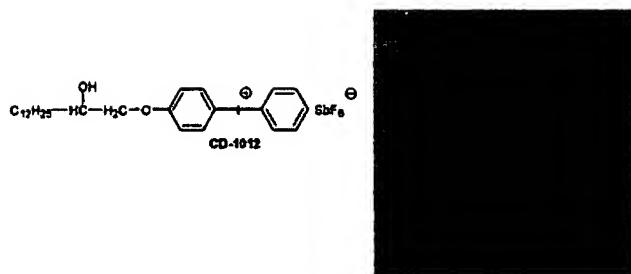
Figure 11. Photoisomerization of fulgide **10**

Figure 9. Optical microscopy image (right) of microstructures (18 μm linewidth, progressively increasing line spacing beginning with 72 μm, from bottom to top) formed via the two-photon cationic polymerization of a mixture of poly(bisphenol A-*co*-epichlorohydrin) glycidyl end-capped and 3,4-epoxycyclohexylmethyl 3,4-epoxycyclohexanecarboxylate (K126, Sartomer) using CD-1012 (left)

triarylsulfonium (CD-1010, Sartomer) salts were found to initiate polymerization of multifunctional epoxide and vinyl ether monomers, affording well-defined microstructures (Figs 9 and 10). Typical multifunctional epoxide monomers investigated were a mixture of poly(bisphenol A-*co*-epichlorohydrin, glycidyl end-capped and 3,4-epoxycyclohexylmethyl 3,4-epoxycyclohexanecarboxylate (K126, Sartomer) or Epon SU-8 (Shell) and K126 in 1:4 weight ratios, respectively, with 1 wt% of either the sulfonium or iodonium initiators. In Fig. 9, the linewidth is 18 μm with progressively increasing line spacing, from bottom to top, beginning at 72 μm (as programmed with the computer-controlled scanner). The microstructure in Fig. 10 has 18 μm linewidths with 72 μm line spacing. The versatility of two-photon polymerization is now well positioned to allow the fabrication of complex three-dimensional microstructures, a prospect we are currently pursuing.

Two-photon photochemical transformations for advanced optical applications

Over the past 50 years, the field of organic photochemistry has produced a wealth of information, from reaction mechanisms to useful methodology for synthetic transformations. Many technological innovations have been
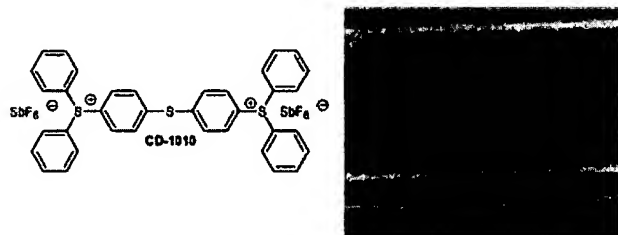

Figure 10. SEM image (right, 250 × magnification) of microstructure (18 μm linewidth, 72 μm line spacing) formed via the two-photon cationic polymerization of Epon SU-8 (Shell) and K126 with CD-1010 (left)
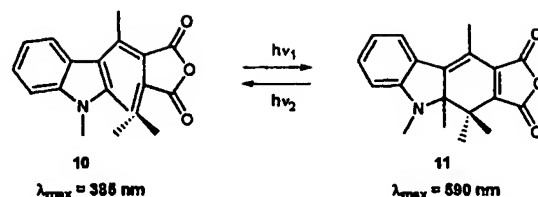
realized during this time due to the exploits of this knowledge, including photoresists and lithography for the production of integrated circuits, photodynamic therapy for cancer treatment, photoinitiated polymerization, UV protection of plastics and humans through the development of UV absorbing compounds and sunscreens, and fluorescence imaging, to name a few. These processes involve 'single-photon' absorption-based photochemistry. Comparatively few studies of multiphoton-induced organic photochemistry have been reported.

In most books on organic photochemistry there is scarcely a mention of simultaneous two-photon induced photochemistry, e.g. in *Electronic Aspects of Organic Photochemistry*, the only mention of this was 'Simultaneous absorption of two photons is also possible and occurs when very high light intensities are used,'[33] while just brief descriptions can be found in *Excited States in Organic Chemistry*[34] and *Principles and Applications of Photochemistry*.[35] This said, the underlying principles of multi- or two-photon absorption are particularly meritorious, and warrant much further investigation (particularly with the advent of commercially available ultrafast pulsed lasers). In fact, the field of two-photon organic photochemistry is in its infancy, not unlike the field of single-photon organic photochemistry 50 years ago.

Rentzepis and co-workers reported two-photon induced photochromism of spiropyran derivatives at 1064 nm.[36,37] Analogous to single-photon absorption facilitated isomerization, the spiropyran underwent ring-opening isomerization to the zwitterionic colored merocyanine isomer. The merocyanine isomer underwent TPA at 1064 nm, resulting in up-converted fluorescence. Spiropyrans are known to undergo photobleaching and photodegradation upon prolonged exposure, and hence are not suitable for long term use. Nonetheless, an intriguing model for 3-D optical storage memory was proposed.

Like many spiropyrans, spirooxazine and fulgide-type compounds are known to undergo photoisomerization from a colorless to highly colored isomer.[38] Unlike the spiropyrans, the thermally and photochemically stable spirooxazine and fulgide-type compounds have been reported which underwent numerous single-photon photochemical isomerization (color) and reversion cycles without significant degradation.[39] Optical data recording potentials as high as 10^8 bits cm^{-2} have been reported for fulgide-type materials. In an effort to develop a more photostable material for two-photon holographic imaging
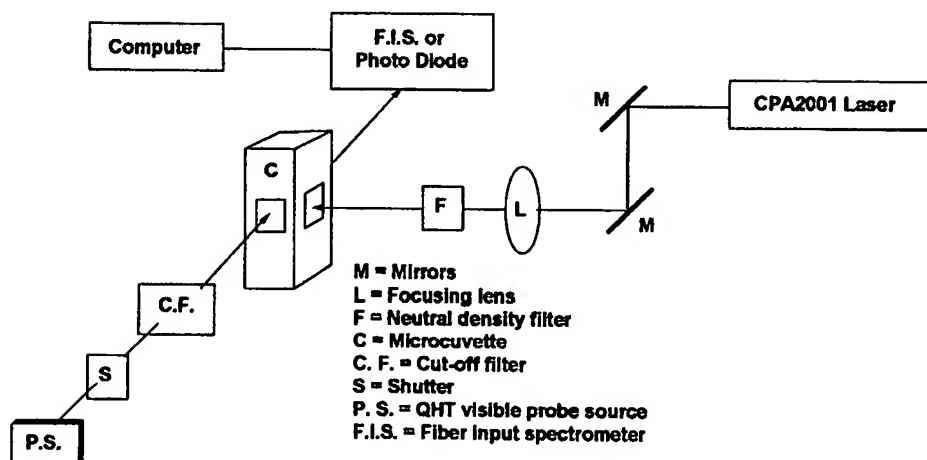
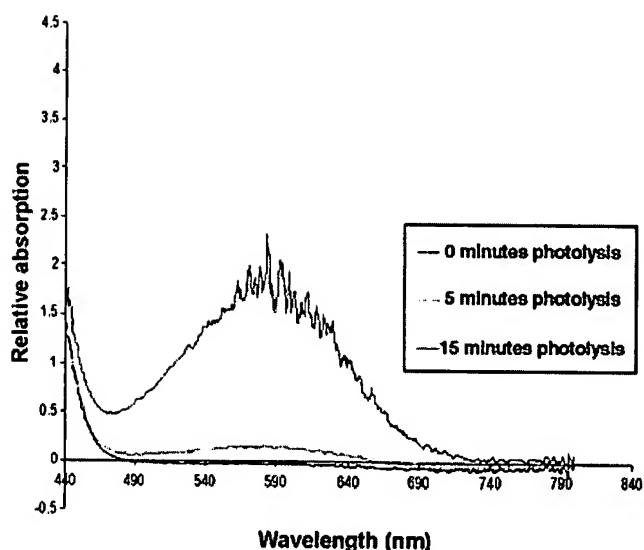**Figure 12.** Optical system for pump–probe photoisomerization kinetics experiment

Figure 13. Absorption spectra as a function of time for the two-photon induced photoisomerization of fulgide **10**

and information storage, fulgide **10** (λ_{max} = 385 nm) was chosen for study, particularly since its single-photon photochromic behavior is well established (Fig. 11).[40]

First, to demonstrate the possibility of two-photon

induced photochromism for **10**, determination of the kinetics of femtosecond near-IR (775 nm) photoisomerization was performed using a pump–probe experimental setup (Fig. 12) to verify the two-photon induced nature of the transformation.

As seen in Fig. 13, formation of the fulgide photoisomer **11** was monitored as a function of time. Plots of absorbance at 585 nm (log I_0/I) versus time (s) were linear for the formation of the ring-closed photoisomer. The photoisomerization rate constants thus obtained were $2.53 \times 10^{-3} \pm 0.3 \times 10^{-3}$ and $6.99 \times 10^{-3} \pm 0.5 \times 10^{-3}\,\mathrm{s}^{-1}$ at irradiation intensities of 3.5 and 7.0 mW, respectively. As can be seen from the rate constants as a function of irradiant intensity, a near-quadratic dependence was observed for the photoisomerization of **10** as a function of intensity of the 775 nm femtosecond pump beam, supportive of a two-photon induced process.

Next, preliminary 2-D interferometric recording was performed using a Mach–Zehnder interferometry setup using a Clark CPA2001 775 nm femtosecond laser as the irradiation source (Fig. 14). Photoinduced changes were observed in the regions of high light intensity (bright interference fringes) in a thin film of poly (styrene)–fulgide **10** composite, demonstrating a proof of principle for effecting photochromic transformations
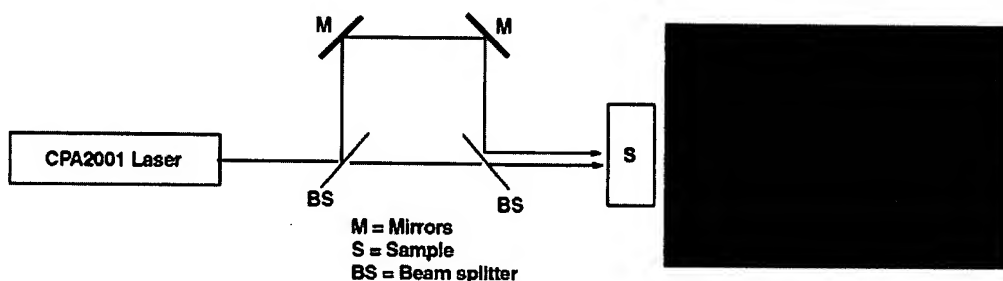

Figure 14. Schematic diagram of a Mach–Zehnder interferometer for 2-D recording via two-photon photochromism (left). Dark lines in right image result from high-intensity bright fringe-induced photoisomerization of fulgide **10** in a polystyrene film (13 μm linewidth and 155 μm line spacing)
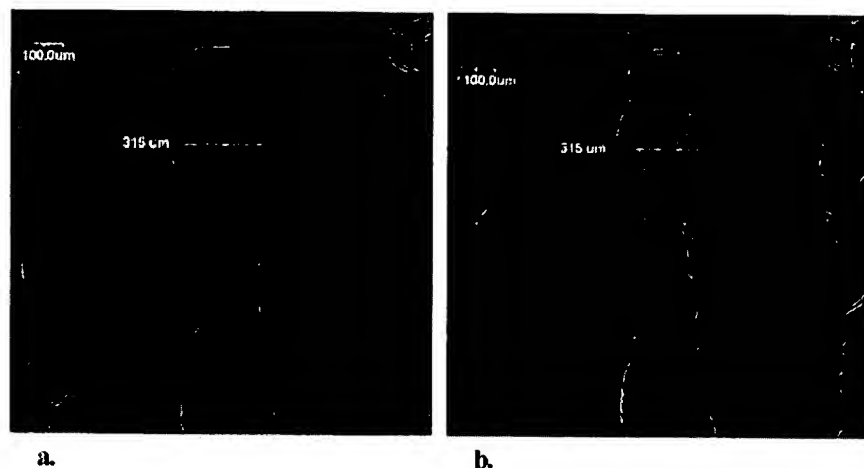
Figure 15. Comparative *xy* (lateral) images of fluorene 6-doped PMMA on scored glass slide via (a) single-photon excitation (Ar, 488 nm) LSCM and (b) two-photon (Ti:sapphire, 815 nm) LSM. Both images were taken from the same focal plane

in localized regions (Fig. 14) as a model for holographic information storage. The dark lines in the image of Fig. 14 result from high intensity bright fringe-induced photoisomerization of fulgide 10 (13 μm linewidth and 155 μm line spacing). Current efforts involve two-photon holographic volumetric recording in this material.

Non-destructive 3-D multiphoton fluorescence imaging

The use of longer wavelength light as the excitation source for fluorescence emission leads to deeper penetration depths than possible with conventional UV or visible fluorescence techniques. Since the fluorescence emission is confined to the focal volume in the two-photon process, there will be virtually no out of focus fluorescence signal, i.e. two-photon excitation falls off rapidly away from the focal volume, resulting in a high signal-to-noise ratio. An advantage of excitation with near-IR radiation is that most materials are transparent in this region. In practice, one is able to image twice as deep in samples using two-photon induced fluorescence compared with conventional confocal single-photon fluorescence microscopy.[9]

For example, use of two-photon fluorescence imaging in the biological sciences ranges from the study of the dynamics of the cytoskeleton in the nematode *C. elegans*,[41] studies of the mechanism by which myoblasts take up DNA,[42] studies of the distribution of mitochondria in developing mammalian embryos,[43] neural transplant processes,[44] 3-D time-lapse imaging of living neurons,[45] lipid membrane dynamics,[46] dendritic spines and other neuronal microdomains,[47] microtubule dynamics in living cells,[48] to the photoactivated release of caged compounds[49] and NADH photoactivation.[50]

Two-photon laser scanning microscopy is a potentially

useful, non-destructive tool to study surfaces, interfaces and fractures in polymer or glass specimens. Non-destructive evaluation refers to a non-invasive technique for probing interior microstructure and subsurface features. Recently, fractures in polymer samples were reportedly imaged by this technique.[51] Two-photon multichannel fluorescence microscopy was also demonstrated to be useful to probe and construct images of multilayered coatings. Figure 15 provides a direct comparision of non-destructive, normal laser scanning confocal microscopic (LSCM) (single-photon) imaging vs two-photon laser scanning microscopic (LSM) imaging of a fluorophore-labeled polymer. Fluorene 6 (Fig. 2) and poly(methyl methacrylate) (PMMA) were dissolved in THF and cast on a scored (scratched) glass substrate.

Comparative lateral (*xy*) images (512 × 512 pixel size, 10 × objective, numerical aperture 0.3) were obtained, whereby both the single-photon (SPE) and two-photon (TPE) excitation of the fluorophore-doped polymer on the glass surface were scanned from the same focal plane within the substrate. SPE was accomplished with a CW argon ion laser (488 nm), and the fluorescence emission was detected by a photomultiplier tube after passage through a 60 μm confocal aperture to reduce out-of-focus fluorescence. Clearly evident in the TPE upconverted fluorescence image (Fig. 15) is the higher contrast resulting from virtually no out-of-focus fluorescence, an inherent manifestation of the TPA process. In two-photon imaging, no confocal aperture was used.

Owing to the quadratic dependence of the TPA process on incident intensity, resulting in minimal out-of-focus emission, a large improvement in emission signal-to-noise ratio is expected. This is borne out in Fig. 16 in which the emission intensity is plotted versus position across the fluorophore-doped polymer in one of the scores [315 μm lines in Fig. 15(a) and (b)]. In addition to an enormous increase in the signal-to-noise ratio, a large
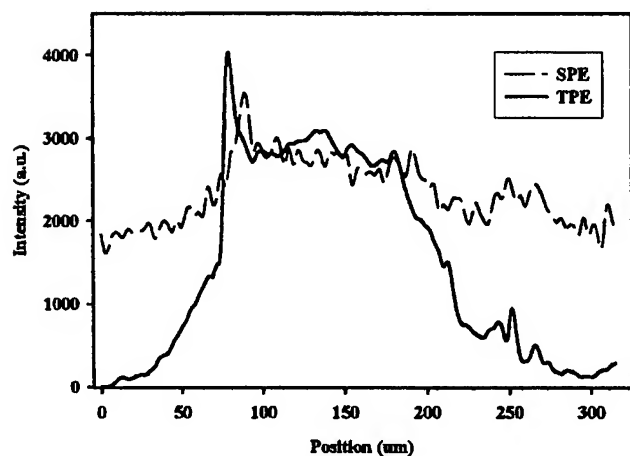
Figure 16. Plots of SPE and TPE emission intensity across the fluorophore-doped polymer in one of the scores [315 µm lines in Fig. 15(a) and (b)]

Figure 18. Plots of SPE and TPE emission intensity across cross-sectioned (xz) images of the fluorophore-doped polymer in one of the scores [335 µm lines in Fig. 17(a) and (b)]

increase in the sensitivity is also observed, as no confocal aperture is used to block emission from the two-photon image.

Both single- and two-photon laser scanning microscopic sectioning can be employed to collect non-destructive cross-sectional images of polymer films. Figure 17(a) and (b) show fluorescence emission across an xy line as a function of depth (5 µm z steps over 340 µm). Figure 17(a) displays the cross-sectional image of the same polymer-coated scored substrate, as shown in Fig. 15, obtained via single-photon laser scanning confocal microscopy (488 nm excitation). In contrast, Fig. 17(b) is the same cross-sectional area imaged via two-photon excitation. Obvious in these images is the substantial increase in resolution, i.e. the single-photon laser scanning confocal microscopic image exhibits out-of-focus fluorescence. The two-photon image is clearly much better defined, providing information on the interfacial micromorphology between the coating and the substrate.

A plot of emission intensity versus depth through the film thickness [along the 335 µm yellow line in Fig. 17(a) and (b)] allows direct comparison of resolution for single- versus two-photon fluorescence imaging (Fig. 18). A remarkable difference is observed with a better resolved signal obtained via TPE, corresponding approximately to the film thickness.

The advantages of two-photon fluorescence imaging of polymer films and interfaces, relative to conventional
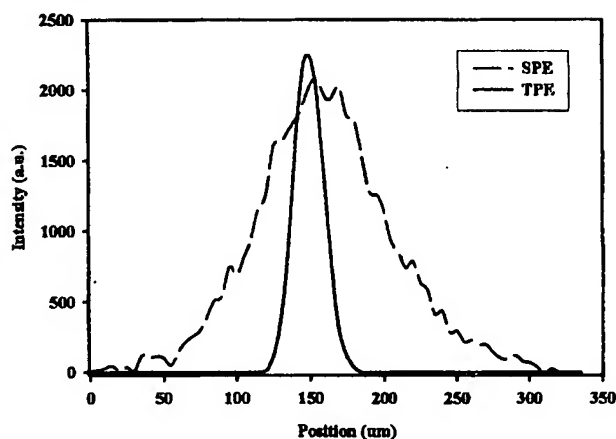
LSCM, are clear. One can anticipate the widespread application of this technique as a non-destructive tool for materials and interfacial micromorphological imaging in the future.

Photodynamic therapy (PDT)

Two-photon activation of photodynamic cancer therapeutic agents should be advantageous since most tissue is transparent to near-IR radiation, affording much deeper penetration depths (allowing subcutaneous treatment). Importantly, the spatial resolution inherent in 2PA will provide increased resolution (localization) in photoactivation of the agent. Psoralen derivatives have been investigated for near-IR femtosecond excitation and have been found to crosslink DNA via $\pi 2s + \pi 2s$ photocycloaddition between psoralen C=Cs and pyrimidine bases of DNA.[52] This results in cell death through inhibiting DNA replication. In addition, porphyrin derivatives have been shown to be effective singlet oxygen sensitizers upon irradiation in vivo (via both single- and two-photon excitation), resulting in tumor cell death.[53] In fact, a porphyrin derivative (Photofrin), approved by the FDA for the treatment of a limited number of cancer types, is gaining increased acceptance in the USA. As PDT becomes a primary therapeutic practice, the advantages associated with highly localized TPA will allow better control of site-specific drug photoactivation.

Figure 17. Cross-sectional (xz) image of the polymer coated glass substrate: (a) single-photon LSCM and (b) two-photon LSM images

In conclusion, the many advantages associated with non-linear absorption-induced processes is fast propelling two-photon absorbing materials to the forefront of several important fields. As the design criteria for increasing multiphoton absorptivity continue to evolve, more efficient TPA organic materials will be sought and prepared. Such materials can be used in 3-D volumetric optical recording, non-destructive 3-D imaging, optical sensor protection, photodynamic therapy and 3-D microfabrication. We can expect to witness breakthroughs in years to come, due to the harnessing of multiphoton absorption in organic materials.

EXPERIMENTAL

Femtosecond optical system for microfabrication and photoisomerization experiments. A laser system (CPA-2001 from Clark-MXR with an Er-doped fiber ring oscillator seeding a Ti:sapphire regeneration amplifier) was used to generate 775 nm light with a 150 fs pulse width at a repetition rate of 1 kHz and ~3 mW average power (~15 µJ per pulse).[14] The beam width was approximately 10 µm. A computer-controlled motorized *xy* step scanner was employed with a scan rate of 1 mm s^{-1}. Monomer/initiator films were spin-coated on glass slides and exposed to the near-IR laser using a variety of scan patterns. Exposed films were imaged using an optical light microscope in reflection mode or by SEM.

Laser scanning microscopy system. The laser scanning microscopy system consisted of an Olympus IX70 inverted microscope and Fluoview confocal laser scanning system fitted with a CW argon ion laser (488 nm, 5 mW) and mode-locked Ti:sapphire laser (80 MHz repetition rate, 120 fs pulse width, 815 nm, 50 mW) pumped with a 5 W frequency-doubled Nd:YAG diode laser (Spectra-Physics Millenia, 532 nm).

Materials. The synthesis and characterization of fluorene derivatives **1–7** are reported in Refs. 8 and 12. The synthesis and characterization of perylenediimide derivatives **8** and **9** are reported in Ref. 15. 2,9-Bis(9,9-didecyl-7-diphenylaminofluoren-2-yl)perylenediimide (**9**): UV–visible (THF): $\lambda_{max} = 261$, 309 and 355 nm (235–415 nm) and $\lambda_{max} = 455$, 488 and 523 nm (415–586 nm). Elemental analysis for $C_{114}H_{124}N_4O_4$: calculates C, 84.82 H, 7.74, N 3.47%; found C, 84.84 H, 7.88 N, 3.33% ^{1}H NMR (200 MHz, CDCl$_3$): δ (ppm) 8.72, 8.70 (d, 4H, ArH), 8.59, 8.55 (d, 4H, ArH), 7.79, 7.75 (d, 2H, ArH), 7.63, 7.58 (d, 2H, ArH), 7.38, 7.36 (dd, 2H, ArH), 7.30, 7.29 (dd, 2H, ArH), 7.27 (dd, 8H, ArH), 7.23 (s, 2H, ArH), 7.16, 7.12 (bm, 4H, ArH), 7.02 (bm, 8H, ArH), 1.90 (bm, 8H, CH$_2$), 1.20 (bm, 28H, CH$_2$), 1.10 (bm, 28H, CH$_2$), 0.90–0.70 (bm, 20H, CH$_2$, CH$_3$). ^{13}C NMR (50 MHz, CDCl$_3$), tentative assignments based on

calculated values: δ (ppm) 163.1 (peryl. C1), 152.5 (C10), 151.8 (C13), 147.9 (C2'), 147.3 (C2), 141.5 (C7), 135.5 (peryl. 18), 134.0 (C12), 132.9 (C11), 131.1 (peryl. 15), 129.1 (peryl. C19), 128.9 (C4'), 128.5 (peryl. C4), 127.5 (C4), 125.8 (C5), 123.8 (peryl. C16), 123.4 (C5'), 122.9 (C1), 122.5 (C3'), 120.8 (C8), 119.7 (C3), 119.2 (C6). FT-IR (KBr, cm^{-1}): 3060, 3033 (ArCH), 2923, 2851 (alCH), 1703, 1665 (C=O). Fulgide **10** was prepared according to a published procedure.[40] All other materials (monomers and photoinitiators) were obtained from commercial sources, as identified in the text.

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